LIMNOLOGY
AND
OCEANOGRAPHY

July, 1960

VOLUME V

NUMBER 3

A STATISTICAL STUDY OF THE ESTIMATION OF ABUNDANCE OF SARDINE (*SARDINOPS CAERULEA*) EGGS[1]

Bruce A. Taft[2]

U. S. Bureau of Commercial Fisheries, Biological Laboratory, La Jolla, California

ABSTRACT

The reliability of the estimation of abundance of sardine (*Sardinops caerulea*) eggs is examined from two points of view. Analysis of egg observations collected on spawning surveys off southern California in 1941 indicates that the counts are consistent with the hypothesis that the egg population is distributed according to the negative binomial contagious distribution. Calculation of 95% confidence limits shows that the cruise estimates are subject to a variation of one-half or double the estimate on the average. A seasonal estimate is probably subject to wider variation than the cruise estimates. Sardine egg observations collected from a 400 square mile grid of 25 stations off Baja California in 1952 over five days show important time and spatial variations. The coefficient of variation of an estimate of abundance of the egg population within the grid for the five day period from a single observation is estimated to be 31.1. The corresponding coefficient of variation of a sum of 40 counts from the grid is 4.9. The implications of this variability for the design of egg sampling surveys are discussed.

INTRODUCTION

Knowledge of the abundance of egg populations serves as a basis for understanding the role of survival in determining recruitment to the adult population (Sette 1943). Egg estimates combined with fecundity data also are employed to estimate the spawning stock size (Clark and Marr 1955). Naturally, the estimation of egg populations is subject to sampling variations. If these estimates are to be used realistically, their reliability must be assessed in order to distinguish between real and sampling variations. In this paper, I will assess the reliability of certain estimates of sardine (*Sardinops caerulea*) egg populations. Basic data used in this study are from the spawning surveys off southern California in 1940 and 1941, jointly conducted by the U.S. Fish and Wildlife Service and the Scripps Institution of Oceanography (Ahlstrom 1948), and unpublished data collected from a special grid of stations occupied off the coast of Baja California in 1952 under the sponsorship of the California Cooperative Oceanic Fisheries Investigations (CCOFI).

The problem of dealing with the statistical errors in estimating plankton populations can be considered from three points of view: 1) How well can we estimate the number of individuals in a sample? 2) How well does the sample represent the volume of water sampled? and 3) How well does this sample from a particular volume of water represent a larger surrounding vol-

[1] The author would like to thank Dr. E. H. Ahlstrom of the Biological Laboratory, La Jolla, for his help at every stage of this research. In the initial phase of the study, the author benefited from many discussions with T. M. Widrig, formerly with the Biological Laboratory, La Jolla. The continued interest and encouragement of John C. Marr, Director, Biological Laboratory, La Jolla, in this study is also appreciated.

[2] Present address: Scripps Institution of Oceanography, La Jolla, California.

ume? Considerable work in the past has been directed to the first two questions (Winsor and Walford 1936; Winsor and Clarke 1940; Silliman 1946; Sette and Ahlstrom 1948; McEwen, Johnson, and Folsom 1954). The importance of the third question has been recognized recently and discussed from a statistical standpoint by Barnes and Marshall (1951) and Holmes and Widrig (1955).

Winsor and Walford (1936) studied various paired vertical plankton hauls and concluded that the distribution of the differences between pairs could be explained by assuming that the organisms were distributed at random and that the hauls were subject only to variability associated with the haul itself. The organisms studied were fish eggs and plankton above a certain size. They concluded that for large numbers the coefficient of variation of hauls was 22.5%. Winsor and Clarke (1940) analyzed repeated hauls in the "same" body of water. The haul to haul variation for oblique hauls of several species of *Calanus* was estimated by a coefficient of variation of 31%. Since they found that the range of variation of the different organisms was roughly proportional to the mean, and that the numbers of different organisms tended to be high or low together, the original data were transformed to logarithms to eliminate the heterogeneity of the error variance. They recommended that comparisons between hauls be made on the logarithms of the counts. Silliman (1946) analyzed sardine egg collections from 24 stations off southern California which were replicated both for sea samples and laboratory samples. At each station two oblique hauls were made, and from each haul two subsamples were taken. Silliman considered only stations where each sample contained more than 15 eggs. To counteract the skewness of his distributions, his original counts were first transformed to logarithms. He concluded that ". . . 90 per cent of the variability in the egg counts is due to real differences in the concentration of eggs at different stations and only 10 per cent to sampling variability." His calculations indicated that a single egg count may be considered statistically significantly different from another if it is less than one-half or more than double the other. He showed that these limits narrow if the mean of a number of hauls is taken. For example, a mean of 150 counts need only show a difference of 7% from another such mean to be considered significantly different. These calculations refer to repeated sampling of the "same" body of water. The reduction of sampling error by repeated observations is usually impractical in a large scale plankton survey program.

Sette and Ahlstrom (1948) considered the problem of estimating sardine egg populations from surveys conducted off southern California in 1940 and 1941. In analyzing the reliability of their estimation procedure they used a statistical model in which each haul was assumed to be a sample from a distinct population with its own mean and variance. A linear interpolation was used between 20 mile stations on the same cruise, and between months at the same station. They estimated the variance of each egg count by using Silliman's figure for the coefficient of variation of haul to haul variability. The variance of a seasonal estimate of egg abundance was calculated by summing the variances of the hauls at each station over all of the cruises within the year. They concluded that their seasonal estimates were subject to an uncertainty arising from the sampling variability of hauls of 5% at the one standard deviation limit. Since their seasonal estimates differed by more than two standard deviations they concluded: "Thus, as far as sampling variabilty of hauls is concerned, the observed difference between the two seasons is demonstratively significant." In a discussion of the errors of interpolation due to wide intervals between stations in space and cruises in time, they concluded from the continuity of the data that there is probably a bias toward systematic underestimation due to the difficulty in assuring that collections are made in the areas of most intense spawning. They did not quantitatively treat the amount of error introduced from letting a

single oblique haul over one-half mile represent a 400 square mile area.

Barnes and Marshall (1951) studied the distribution of certain constituents of the plankton by sampling from a single depth over several hours while the ship was drifting relative to the water. They were measuring short distance spatial and short period time variations. Organisms studied were various copepod nauplii, lamellibranch larvae, and unidentified eggs. The samples were collected by a plankton pump, thus virtually eliminating variations in the amount of water sampled. They concluded that the organisms were not distributed at random except at low population densities. They used the Neyman Type A and the Thomas contagious distributions to describe the variability. (A contagious distribution is a distribution in which it is assumed that the occurrence of an organism in an area increases the probability of finding other like organisms.) They suggested that the results of Winsor and Walford (1936) could be adequately explained on the hypothesis that the organisms were distributed in a non-random manner.

Holmes and Widrig (1955) studied the problem of enumeration of phytoplankton in the laboratory and the apparent distribution of phytoplankton in the sea. Their data indicated that contagion existed for the organisms studied. They discussed the difficulties of comparing different areas of the sea with respect to their plankton populations when the organisms exhibit a variability characteristic of a contagious series. They conclude: ". . . with only a single sample in an area one cannot describe a population very accurately, either with respect to composition or abundance. Thus, if we want to characterize an area, we must take more than one sample at random from within that area."

In this paper two approaches to the problem of assessing the reliability of sardine egg estimates will be used. First, the eggs collected in the 1941 spawning survey off southern California will be considered to be drawn from a contagiously distributed population. A particular contagious distribution, the negative binomial, will be proposed to describe this egg distribution in the sea. The implications of this statistical model for sampling will then be discussed and confidence limits for the population estimates written out. Second, data from a special grid of stations will be analyzed to estimate the magnitude of short period (one day) time changes and the effect of the spatial distribution of sardine eggs within a 400 square mile area of the ocean off Baja California. An analysis of the variance will yield estimates of the components of variance attributable to spatial stratification and variation in time in estimating the population within the grid from a single station.

COMPUTATION OF EGG POPULATION ESTIMATES

The computation of estimates of the sardine egg population is discussed in Sette and Ahlstrom (1948). The raw count derived from a complete count of a plankton collection, or from a known proportion of a collection, is adjusted to give the number of eggs spawned per day per 10 m^2 of sea surface. The standardization of the counts with respect to volume of water strained is accomplished by placing a current meter in the mouth of the net and calculating the volume of water strained from the observed average number of revolutions per second (Ahlstrom 1948). The count is then converted to the number of eggs collected per 10 m^3 of water strained per unit of depth sampled. This is equivalent to the number of eggs per 10 m^2 of sea surface. The adjustment of the data to represent the number of eggs under a standard area (10 m^2) introduces errors into the calculation in addition to those associated with the laboratory sampling and the operation of the net in the water. These errors have not been analyzed but may be presumed to have only a small effect since Silliman (1946) concluded from a study of adjusted and unadjusted egg counts that the adjustments did not significantly affect the variability. Errors inherent in the method of collection are apparently large compared to errors introduced by adjustment. The virtue of the adjustment is that it makes possible an areal estimate of egg abundance. The basic

variable studied here will be the number of eggs per 10 m^2 of sea surface.

Since the number of eggs found at any one time in the sea is influenced by their rate of embryonic development, it is desirable to estimate the average number of eggs spawned per day. This quantity is estimated by dividing the total number of eggs in a standardized haul by the number of day's spawning represented. The number of day's spawning is determined by the relationship between rate of development and temperature (Ahlstrom 1943, 1954).

$$\log_{10} h = 1.809 - 0.0558\ (t - 15.40)\ ,$$

where h = estimate of average number of hours from spawning to hatching and t = temperature in °C at 10 m depth.

A single haul is taken to represent a certain area for a time interval determined by the interval between successive occupancies of the station. The area for each station is determined by the perpendicular bisectors of the lines connecting adjacent stations. An estimate of the population size for a cruise is

$$C_{i\cdot} = D_i \sum_{j=1}^{n_i} \frac{A_{ij}\, c_{ij}}{d_{ij}} \qquad (1)$$

where $C_{i\cdot}$ = estimate of egg population for *i*th cruise, A_{ij} = number of standard area (10 m^2) units at *j*th station, D_i = period represented by the *i*th cruise in days, d_{ij} = estimate for the *j*th station of the time in days from spawning to hatching, n_i = number of stations occupied during *i*th cruise, and c_{ij} = total number of sardine eggs per 10 m^2 of sea surface at *j*th station on *i*th cruise. The factor D_i is equal to the number of days of the cruise plus one-half the days intervening between the end of $i-1$th cruise and the start of the *i*th cruise plus one half the number of days between the end of the *i*th cruise and the start of the $i + 1$th cruise.

A simplified formula for calculating the estimate is

$$C'_{i\cdot} = \frac{A_{i\cdot}\, D_i}{d_{i\cdot}} \sum_{j=1}^{n_i} c_{ij} \qquad (1a)$$

where $d_{i\cdot}$ = estimate for period of cruise of the average number of days from spawning to hatching and $A_{i\cdot}$ = mean number of standard area (10 m^2) units in survey area per station.

Sette and Ahlstrom (1948) show in their calculations that the seasonal estimate of abundance using the above simplified cruise estimates was within 4% of the seasonal estimate for both years using development data and area factors for each station. The estimate $C'_{i\cdot}$ is an unweighted sum of the counts and therefore lends itself to statistical treatment much more readily than $C_{i\cdot}$.

The calculated variance of $C_{i\cdot}$ on the assumption of random sampling is

$$\begin{aligned} S^2_{C_{i\cdot}} &= D_i^2 \sum_{j=1}^{n_i} A^2_{ij}\, S^2_{\frac{c_{ij}}{d_{ij}}} \\ &\doteq D_i^2 \sum_{j=1}^{n_i} A^2_{ij} \left[\frac{c^2_{ij}}{d^2_{ij}} \left(\frac{S^2_{c_{ij}}}{c^2_{ij}} + \frac{S^2_{d_{ij}}}{d^2_{ij}} \right) \right] \\ &\doteq D_i^2 \sum_{j=1}^{n_i} A^2_{ij} \left[\frac{c^2_{ij}}{d^2_{ij}} \left(V^2_{c_{ij}} + V^2_{d_{ij}} \right) \right], \end{aligned} \qquad (2)$$

where $V^2_{d_{ij}}$, $V^2_{c_{ij}}$ are respectively the relative variances of d_{ij} and c_{ij} . This formula holds for $V_{d_{ij}} \leq 0.05$ (Hansen, *et al.* 1953, Vol. II, pp. 109–111). An estimate of $V_{d_{ij}}$ may be obtained from the regression of time of development on temperature. Even at the temperature extremes of observed sardine spawning, where the error of estimation of the time from spawning to hatching is the greatest, the condition is easily satisfied. For example, at a temperature of 13°C or 17.8°C, the coefficient of variation of d_{ij} is 0.02.

The question that naturally arises is the relative magnitude of $V^2_{c_{ij}}$ and $V^2_{d_{ij}}$. Silliman (1946) estimated the variance of the logarithms of the haul counts to be 0.031. This corresponds to a coefficient of variation of c_{ij} of 0.42. A comparison of this figure with the above coefficient of variation d_{ij} clearly indicates that the coefficient of variation of d_{ij} is negligibly small relative to that of the hauls themselves. The expres-

sion for the calculated variance of a cruise estimate is then

$$S^2_{C_i\cdot} = D_i^2 \sum_{j=1}^{n_i} \frac{A_{ij}^2 S^2_{c_{ij}}}{d_{ij}^2} . \quad (3)$$

For the simplified estimate $C'_{i\cdot}$ the variance is

$$S^2_{C'_i\cdot} = \frac{A_{i\cdot}^2 D_i^2}{d_{i\cdot}^2} \sum_{j=1}^{n_i} S^2 c_{ij} . \quad (3a)$$

A seasonal estimate of the abundance of eggs, $C_{\cdot\cdot}$, is a summation over the r cruises during the season.

$$C_{\cdot\cdot} = \sum_{i=1}^{r} C_{i\cdot} = \sum_{i=1}^{r} D_i \sum_{j=1}^{n_i} \frac{A_{ij} c_{ij}}{d_{ij}} . \quad (4)$$

The calculated variance is accordingly

$$S^2_{C_{\cdot\cdot}} = \sum_{i=1}^{r} D_i^2 \sum_{j=1}^{n_i} \frac{A_{ij}^2 S^2 c_{ij}}{d_{ij}^2} . \quad (5)$$

The analogous expression for the variance of the summation of the simplified cruise estimates is

$$S^2_{C'_{\cdot\cdot}} = \sum_{i=1}^{r} \frac{A_{i\cdot}^2 D_i^2}{d_i^2} \sum_{j=1}^{n_i} S^2 c_{ij} . \quad (5a)$$

I assume that each month's survey constitutes a random sample of eggs from the survey area. The different cruises are then considered to be samples from distinct egg populations. In the language of survey sampling, each monthly egg collection may be regarded as a random sample from a single time stratum. This model is based on the assumption that there are real differences in the spawning between months, *i.e.* there is a trend in sardine egg spawning during the season but that there is no trend within a month. Since the division of the time according to cruise is purely arbitrary, there is no assurance that fluctuations within the period of the cruise are strictly chance fluctuations. Possible bias introduced into the estimation from this source is not evaluated. Since the stations are not usually chosen purposively with respect to the egg population, this distortion may not be very great.

ANALYSIS OF SARDINE SPAWNING SURVEYS OF 1940–1941

In the spring and early summer of 1940 and 1941 a grid of stations was occupied off southern California at monthly intervals. One of the purposes of these cruises was to provide an estimate of the abundance of sardine egg populations in this region. Oblique plankton hauls to approximately 75 m were made at each station and eggs enumerated (Ahlstrom 1948). These hauls can be presumed to sample the vertical distribution of sardine eggs representatively since egg collections from vertical series show that the eggs lie above 75 m (Ahlstrom 1959). The area extended from Point Dume to a point midway between San Diego and Ensenada, and extended approximately 150 miles seaward. During 1940, six cruises occupied from 31 to 42 stations per month. Forty-one stations were occupied on each of the six cruises during 1941. Stations were spaced approximately 20 miles apart.

In choosing these areas to study the distribution of sardine eggs, I am not dealing with the total population of eggs. The range of spawning no doubt extends beyond the block of stations occupied during these cruises (Ahlstrom 1954). The population estimates and their reliability would no doubt have changed if the total range of spawning had been included in the survey. In any case where the total spawning was covered, the definition of the population would also pose a problem because the absence of any organisms at a particular station could not necessarily be interpreted as delimiting the population sampled. The determination by the absence or presence of eggs of the spatial range of the population is also dependent on the volume of the sampling unit. The selection of the range of the egg population would always be somewhat subjective and the estimated reliability would be dependent on the number of zero observations included in the range of spawning. The fitting of an exact

TABLE 1. *Frequency distributions and fitted negative binomial distributions of sardine eggs per 10 m² of sea surface for 1941 cruises 17, 19, and 21*

Cruise	c_{1j}	f	ϕ	χ^2	
17	0–74	8	5.05	1.72	
	75–199	9	5.14	2.90	
	200–399	5	6.00	0.17	
	400–699	7	6.37	0.03	
	700–1099	5	5.74	0.10	
	>1100	7	12.70	2.56	
				7.48	$<\chi^2_3(.05)=7.81$
19	0–2	7	5.02	0.78	
	3–20	8	5.00	1.80	
	21–75	6	5.48	0.05	
	76–199	4	5.83	0.57	
	200–400	3	5.02	0.81	
	401–800	6	5.23	0.11	
	>800	7	9.42	0.62	
				4.74	$<\chi^2_4(.05)=9.49$
21	0	14	14.43	0.01	
	1–5	6	5.16	0.14	
	6–28	8	5.04	1.74	
	29–149	3	6.46	1.85	
	>150	10	9.91	0.00	
				3.75	$<\chi^2_2(.05)=5.99$

mathematical distribution to such subjective data may be questioned (Gulland 1956). Only on cruise 19 in 1941, where five out of six stations with zero counts are inshore, is there a suggestion that the survey region was broader than the region occupied by sardine eggs. The general region during the years 1940–1941 was the scene of consistently heavy spawning during the spring months.

An examination of the frequency distribution of sardine egg counts for 1941 cruises 17, 19, and 21 in Table 1 shows a characteristically large number of stations with low counts in conjunction with a smaller number of stations with very high counts. The mean level of the counts for a cruise is largely influenced by the presence of a few very high counts. The counts in all cases vary over three orders of magnitude. The problem of obtaining a representative sample of the egg population resides in including the stations with very high counts in proportion to their true frequency in the population. With a not very dense network of sampling stations the probability of inclusion of these very high egg count stations is obviously low. Under these conditions, the level of the estimate is very likely a function of the density of the stations.

TABLE 2. *Means and variances of sardine eggs per 10 m² of sea surface for 1940–1941 cruises*

Cruise	Number of stations	Mean	Variance	Variance / Mean
1940:				
10	37	726	890,373	1,226
11	31	906	1,729,000	1,908
12	33	569	570,006	1,002
13	40	1,386	8,461,959	6,105
14	42	863	3,085,128	3,575
15	40	59	23,428	397
1941:				
17	41	976	3,525,823	3,613
19	41	605	2,262,431	3,740
21	41	243	257,915	1,061
23	41	76	45,812	603

It is well known from sampling theory that if there is a relatively small number of organisms per unit volume of water sampled and that if the organisms are distributed at random throughout the area sampled, the organism counts per unit volume (area) will follow a Poisson distribution. The variance of this distribution is equal to its mean. The means, variances and their ratios for the 1940 and 1941 cruises are presented in Table 2. The ratios of variance to mean vary from 397 to 6,105. These data clearly indicate that we are dealing with a nonrandom distribution of sardine eggs. Plots of the variance of the cruise counts as a function of their mean for 1940 and 1941 are shown in Figures 1 and 2. Freehand curves are drawn to emphasize the trend. The plots for both years have a curvature suggestive of a parabolic curve symmetric to the ordinate axis. The slope indicates that the variance is correlated with the mean in such a way that the variance is increasing at a greater rate than the mean, which implies that the mean egg density is varying within the survey region. Thus we can think of a volume of the sea containing a number of populations or patches of sardine eggs

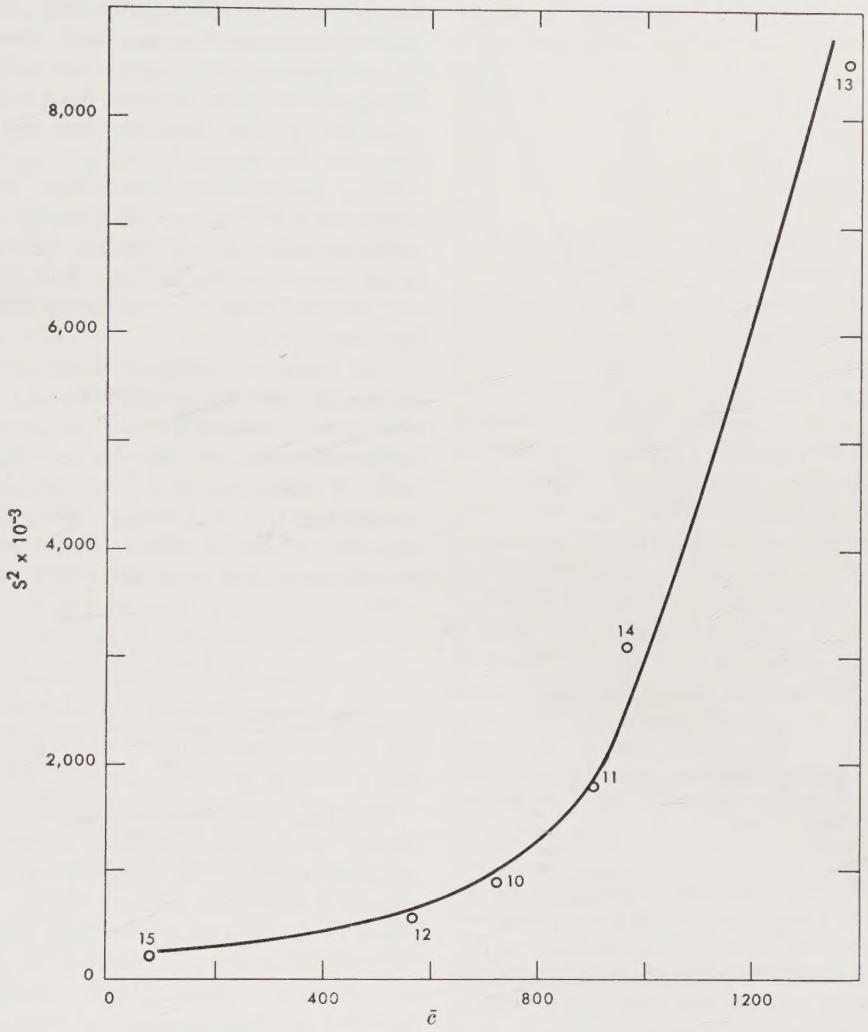

FIG. 1. Relation between mean ($\bar{c}$) and variance (S^2) of sardine eggs per 10 m^2 of sea surface for 1940 cruises 10 to 15.

with different mean densities. The variance of a set of counts from such a region will be the sum of the variance within a patch and the variance of the mean between patches. If we assume that the variation within a patch of eggs is Poisson, then the variance within a patch will be equal to the mean. If, in addition, we assume that the variance of patch means is proportional to the square of the over-all mean, then the expression for the variance of a count can be written in the form

$$\sigma_c^2 = m + \frac{m^2}{K} \quad , \qquad (6)$$

where m is the mean number of eggs per 10 m^2 of sea surface and K is a coefficient that expresses the relative contributions of within-patch variability and between-patch variability. As the variation of mean level between patches increases, *i.e.* the contagion of the organisms increases, the magnitude of K will decrease and the term $\frac{m^2}{K}$ will contribute more to the total variance. Equation (6) is consistent with the shape of the plots of the variance against the mean in Figures 1 and 2 if K is presumed to be constant over the cruises.

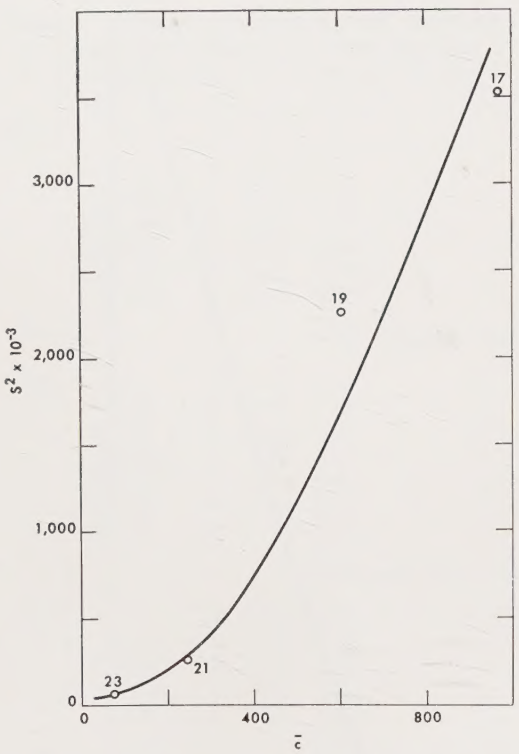

FIG. 2. Relation between mean ($\bar{c}$) and variance (S^2) of sardine eggs per 10 m^2 of sea surface for 1941 cruises 17 to 23.

The negative binomial contagious distribution has a variance of the form proposed in (6) and the data were examined to determine whether this distribution adequately described our sardine egg counts. Once the negative binomial form of our frequency distributions is established, then a transformation of the counts is available to yield a variable whose distribution can be considered approximately normal. This transformed variable can then be used to write out the confidence limits of an egg estimate. Other unimodal contagious distributions which might have fitted the data equally well could have been examined, but the negative binomial was chosen because of the extensive literature on its sampling properties.

No attempt will be made to justify logically the choice of the negative binomial frequency function with regard to the underlying population relationships. Data on the distribution and aggregation of spawning sardines and the resulting distribution of egg patches does not exist at present. The point of view adopted here is that the variation in egg density over the survey area can be shown to be not inconsistent with a logarithmic distribution of patch means and a Poisson distribution of eggs within a patch, which implies that the eggs in the survey region are distributed according to the negative binomial frequency function.

The negative binomial distribution is a unimodal contagious distribution and is completely defined by two parameters, the arithmetic mean m and the positive coefficient of contagion K. The probability of occurrence of c individuals drawn from a negative binomial distribution with mean m and contagion coefficient K is

$$P_c = \frac{(K + c - 1)\,!\,R^c}{c\,!\,(K-1)\,!\,q^K}$$

where $R = \frac{m}{m+K}$, $q = \frac{1+m}{K}$, and and $c = 0, 1, 2, \ldots$.

The negative binomial has two limiting forms. As the coefficient of contagion approaches infinity, the negative binomial approaches the Poisson distribution. As the value of K approaches infinity expression (6) reduces to $\sigma^2_c = m$ which is the variance of a Poisson variate. If the contagion coefficient approaches zero, *i.e.* contagion increases, then the negative binomial converges to Fisher's logarithmic series when the zero class is ignored. Under these conditions the variance approaches $\frac{m^2}{K}$.

Negative binomial distributions were fitted to the first three cruises of 1941 (17, 19, 21). During these cruises 96% of the eggs collected on all six cruises were obtained. Data from the 1940 collections were not used because of the lack of a repeated pattern for all cruises. This changed the area weights within a cruise and precluded straightforward statistical treatment of the distribution of eggs. The maximum likelihood estimation of K has been worked out

by Fisher (1953), but his method is impractical for very high means. Data from cruises 17 and 19 were fitted by a procedure described by Anscombe (1949). The counts are transformed by

$$y_{ij} = \log_{10}(c_{ij} + \tfrac{1}{2}K). \qquad (7)$$

This transformation is applicable for high means (>15) and low K (<1). Under these conditions the expected variance of y_{ij} is distributed approximately independently of the mean and equal to 0.1886 ψ' (K), where ψ' (K) is the trigamma function of K. Values of the trigamma function are given by Davis (1933). The procedure is to guess a value of K, calculate the variance of y_{ij} and then compare it to 0.1886 ψ' (K). This process is continued until satisfactory agreement is obtained. The efficiency of this method of estimation of K and its sampling variance are not known. Data from cruise 21 were fitted by solving the equation

$$K \log_{10}(1 + \bar{c}_{i\cdot}/K) = \log_{10} n/f_o \qquad (8)$$

for K, where n is the total number of counts, f_o is the number of zero observations and $\bar{c}_{i\cdot}$ is the mean count. This estimation is at least 90% efficient as compared with maximum likelihood estimation if more than one-third of the counts are zero. This condition holds for cruise 21, but not for cruises 17 and 19. The large sample variance of K as estimated from equation (8) is given by Bliss (1953).

$$S_K^2 = \frac{(1-R)^{-K} - KR - 1}{n\left[-\log_e (1-R) - R\right]^2} \qquad (9)$$

The distributions and their fitted negative binomial distributions are given in Table 1. The hypothesis of a negative binomial distribution was tested by the χ^2 goodness of fit test.

$$\chi^2 = \sum_i \frac{(f_i - \phi_i)^2}{\phi_i}$$

where ϕ_i is the calculated or hypothetical frequency and f_i is the observed frequency. The computed value of χ^2 was compared with χ^2_{m-3} for the 5% level. In all cases the hypothesis of an underlying negative binomial distribution was accepted at the 5% level of significance. Because of the length of the computation, probabilities were not calculated for every successive count past 50. The probability was calculated at 25, 50 or 100 count intervals and a linear gradient of the frequencies in the interval was assumed in order to estimate the total area under the probability function between the two points. The error in estimated frequency is less than 1% and can be presumed not to affect the conclusions of the tests of goodness of fit. The estimated K's for the three cruises are found in Table 3. S(K) is the standard error of K estimated from (9). There is an apparent correlation of the contagion parameter with the mean level. As the mean density of organisms drops, the relative variation in mean between patches apparently increases. With only three pairs of values this relationship cannot be firmly established.

TABLE 3. *Estimated K's, standard error of K [S(K)] and means ($\bar{c}_{i\cdot}$) for cruises 17, 19, and 21*

Cruise	K	S(K)	$\bar{c}_{i\cdot}$
17	0.76	—	976
19	0.34	—	605
21	0.14	.047	243

Anscombe (1948, 1949) has indicated that the variable

$$y_{ij} = \log_{10}(c_{ij} + \tfrac{1}{2}K)$$

can be treated as a normally distributed variable with error variance 0.1886 ψ' (K) for K>5 and high mean. Also, it has been shown that if a variable has a negative binomial distribution with contagion parameter K, the sum of n counts from such a population will have a negative binomial distribution with contagion parameter nK (Anscombe 1949). With this information it is possible to write out the confidence limits for the total of n counts. The confidence limits for a given probability level α are

$$\text{antilog}_{10}\left[\log_{10}\left(c_{i\cdot} + \frac{nK}{2}\right) \pm t_\alpha \sqrt{0.1886\,\psi'(nK)}\right] - \frac{nK}{2}, \qquad (10)$$

TABLE 4(a). *Estimates of total sardine eggs per 10 m² of sea surface and 95% confidence limits of the estimates*

Cruise	Upper 95% limit	Estimate	Lower 95% limit
17	57,024	40,015	28,074
19	42,443	24,804	14,443
21	23,277	9,974	4,277

TABLE 4(b). *Estimates of total number of sardine eggs spawned into the survey region and 95% confidence limits of the estimates (all numbers* $\times 10^{-9}$)

Cruise	Upper 95% limit	Estimate	Lower 95% limit
17	102,073	71,627	50,252
19	67,484	39,438	22,964
21	41,433	17,754	7,613

where t_a is taken from a table of the standardized normal variable such that $1-a$ of the distribution falls within $\pm t_a$. The quantity $0.1886\ \psi'$ (nK) is the expected variance of the transformed total count.

The estimates of the number of eggs collected on cruises 17, 19 and 21, and the computed 95% confidence limits are given in Table 4(a). These limits are calculated such that 95% of the time they will include the true abundance of the eggs. Corresponding estimates of the number of eggs spawned in the area of survey, using formula 3(a), are given in Table 4(b). Formula 1(a) was used to calculate the estimates, since the distribution function appropriate to a weighted sum of counts with a negative binomial distribution is unknown. Since the area, time and rate of development weights are relatively uniform, it is presumed that conclusions drawn about $C'_{i.}$ are also applicable to the more accurate estimate $C_{i.}$. Because of the approximate normality of the logarithmic transformation these limits are symmetrical on a log scale and multiples of the estimate on the original scale. Multiplying factors, *i.e.* the ratio of upper limit to estimate, vary from 1.4 for cruise 17 to 2.5 for cruise 21. The limits are narrowest in relative terms for cruise 17 where contagion as measured by K was least, and widest for cruise 21, where the contagion was the greatest. It is clear from the confidence limits that the number of eggs spawned differed significantly from one cruise to the next as in no case do the confidence limits for one cruise's estimate include an estimate for another cruise. In order to compare the level of spawning between cruises for other situations where the eggs show comparable degrees of contagion, the ratio of estimates would have to exceed 1.4 at high densities and 2.5 at lower densities to be quite sure that the fluctuations were real and not due to sampling errors. To perform an actual test of significance of the difference between two estimates a t-test, or some variation of it to take into account differing variances, would have to be used.

So far we have only dealt with estimates from a single cruise, and the question naturally arises as to the error in the estimation of the spawning over a season's time. The analysis of these egg distributions indicated that the distribution itself, in addition to the means, varied significantly between months. The values of K varied from 0.14 to 0.76 and therefore they could not be considered homogenous. The only estimate of K for which the sampling variance could be written indicated that the K = 0.14 was significantly different from the others. Since the distribution is not homogenous with respect to K over the three cruises, the data cannot be pooled to estimate a common K. The distribution of the sum of sets of counts from different negative binomial distributions is unknown, and consequently the problem of the reliability of a seasonal estimate cannot be treated statistically, and any statement will have to be qualitative. The variance of a seasonal estimate is given by (5a), which is the sum of the variances of the cruises within the season. The absolute error of estimate for the seasonal estimate will therefore increase. However, the relative error as measured by the relative variance will decrease for the seasonal estimate of abundance. The formula for the relative variance of a sum $C'_{..}$ of independent variables $C'_{i.}$ is

$$V^2_{C'_{..}} = \frac{1}{(C'_{..})^2} \sum_{i=1}^{r} (C'_{i.})^2 \, V^2_{C'_{i.}} \quad .$$

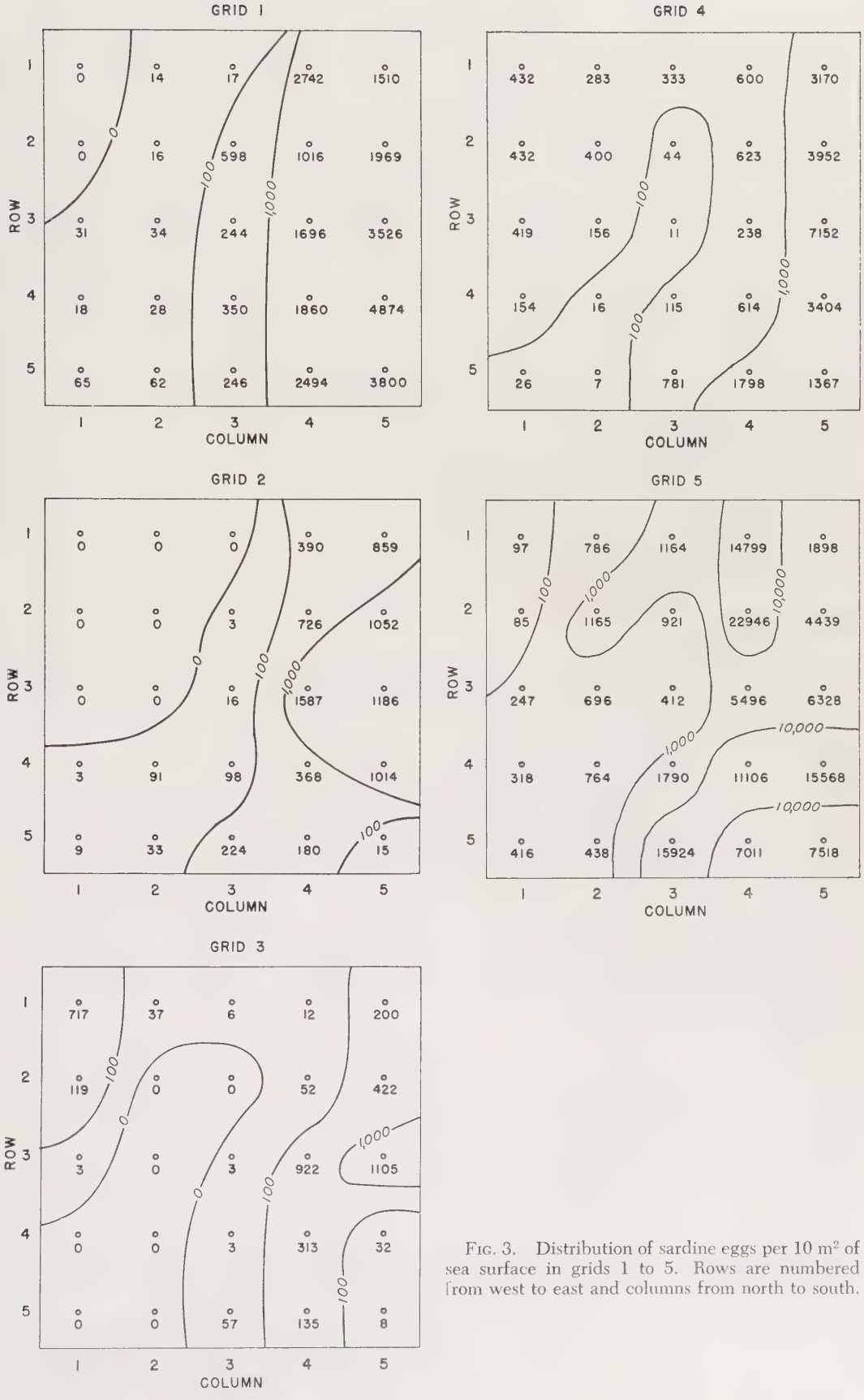

FIG. 3. Distribution of sardine eggs per 10 m^2 of sea surface in grids 1 to 5. Rows are numbered from west to east and columns from north to south.

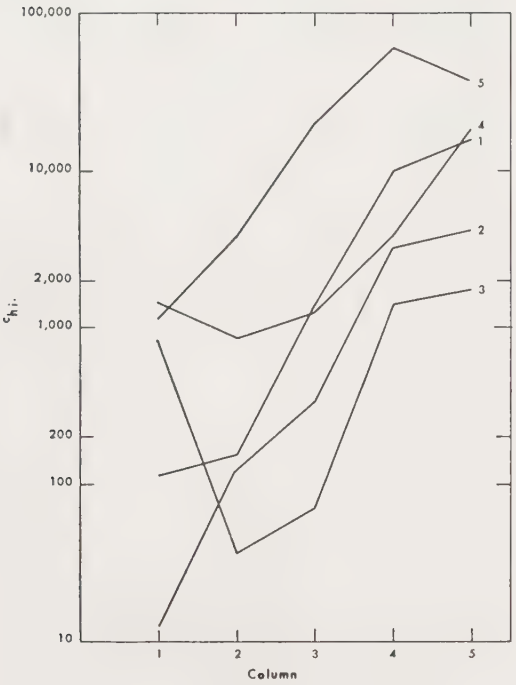

FIG. 4. Plot of column totals ($c_{hi.}$) against column position for grids 1 to 5.

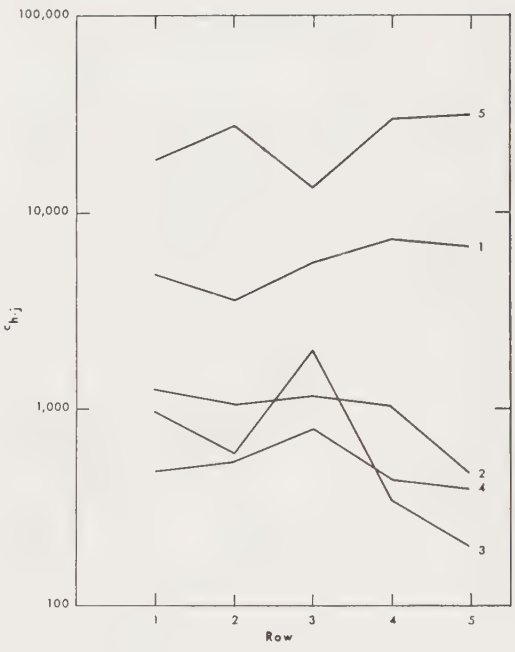

FIG. 5. Plot of row totals ($c_{h.j}$) against row position for grids 1 to 5.

For a seasonal total of the 1941 collections $V^2_{C'..}$ is 0.042, which is less than any of the constituent relative variances. This estimate of error is minimal since it assumes sampling of a stable population. In our case, the cruises are taken to represent approximately a month's period during which the population is subject to considerable fluctuation. Thus there is a component of variance from this source that remains unevaluated. A seasonal estimate for this reason probably is not more precise in relative terms than the individual cruise estimates, and could be considerably less precise.

ANALYSIS OF GRID OBSERVATIONS

In 1952 a series of closely spaced oblique net tow observations to approximately 140 m were taken to study spatial and temporal variations of the population(s) of sardine eggs. The stations were spaced four miles apart, and a square grid of 25 stations was occupied on five successive days. This grid was centered off central Baja California at approximately 27° 12′N, 115°03′W. Each day's observations commenced at 0600, when the contribution to all egg stages of the previous night's spawning can be assumed to be virtually complete (Ahlstrom 1943).

The orientation of the isopleths of egg abundance per 10 m^2 of sea surface shows that the eggs tended to be distributed in a NW-SE direction over the period of the survey. These isopleths are drawn in Figure 3. The eastern portion of the grid consistently shows higher egg counts than the western portion. The rows (E-W sections) and columns (N-S sections) are numbered on Figure 3 to facilitate discussion. The column and row totals are plotted in Figures 4 and 5 on a log scale against position. The gradient of total abundance from west to east (Figure 4) is reasonably constant except between columns 1 and 2. Between columns 1 and 2, the gradient is not consistently positive or negative. This section is also the section of lowest egg counts. For the sections to the east of column 2, the gradient is positive except for the section between columns 4 and 5 of grid 5. East

to west section (row) totals are plotted in Figure 5. There is no dominant trend in this data, and it presents a much more variable picture. A slight negative gradient is discernible in three of the grids from row 3 to row 5. In only one case (grid 3) is there a change of an order of magnitude among rows. Among columns the variation is between two and three orders of magnitude. Thus, it is clear that these populations of eggs are relatively homogenous in a direction parallel to the coast, but that an intense offshore gradient exists. In conjunction with this grid study, a subsurface buoy placed at 10 m depth was followed over the period of the grid study. The track of this buoy showed a consistent current of about 0.6 kn bearing from slightly west of north. This observed pattern of egg density gradients then represents a gradient in the intensity of spawning within and to the north of the grid.

Even though the within-grid pattern shows consistency over the five days of sampling, the level of abundance varied widely. The totals vary from 4,146 to 122,332 eggs per 10 m^2 of sea surface. The ratio of highest day's total collection of eggs to lowest is approximately 30. Another way of expressing the wide variations of egg counts encountered in the various grids is that five stations in grid 5 accounted for 80,343 eggs, whereas the total collected on the four previous grids was 65,739. These variations represent day to day variations in the intensity of spawning within and to the north of the grid.

Since the egg populations were not homogenous over the five days of sampling, it is necessary to treat each day's sampling as drawn from a different population. The severe stratification noted in the analysis of N-S sections of the grid indicates that the variation within a day's grid is not random but consistent in pattern. Consequently, the population cannot be considered homogenous within the grid itself. The statistical model to be applied to the analysis of these observations will presume that the spatial and temporal differences observed here are real and their contributions to the total variance will be estimated.

In order to estimate the variability arising from various sources, it is necessary to stabilize the variability so that it is constant from one sampling unit to the next. I will consider each observation as a sample of one from a population defined by its position in the grid (row and column) and the day's grid. Since our observations are classified according to three criteria and there is no replication of individual cell entries, an analysis of the stability of the error variance is not possible. However, the variances and means of the different grids provide an opportunity to observe a correlation of variance and mean. The means, standard deviations and total number of eggs for the five day's grids are found in Table 5. The means and standard deviations are plotted in Figure 6. The plot indicates that the standard deviation is proportional to the mean or that

$$\sigma^2 = p^2 m^2 \quad ,$$

where p^2 is a constant of proportionality. It will be assumed that this relation also holds for the individual hauls (Sette and Ahlstrom 1948).

In order to stabilize the variance it is necessary to find a transformation, $y = g(c)$, to yield a variable with a variance independent of the mean level. Bartlett (1947) states that for any function $g(c)$, $\sigma^2_{g(c)}$ is approximately related to $f(m)$ by

$$\sigma^2_{g(c)} = \left(\frac{dg}{dm}\right)^2 f(m) \quad . \qquad (11)$$

Thus we can set $\sigma^2_{g(c)}$ equal to a constant K^2 and solve for $g(m)$ letting $f(m) = p^2 m^2$.

$$g(m) = \frac{K}{p}\int \frac{dm}{m} + \text{const.} = \frac{K}{p} \log m + \text{const.}$$

Thus a logarithmic transformation of the plankton counts should yield a new variable y, whose variance is independent of the mean. In order to handle the zero counts, one will be added to all the counts before taking their logarithms. A plot of the standard deviation of the logarithms of the counts plus one as a function of

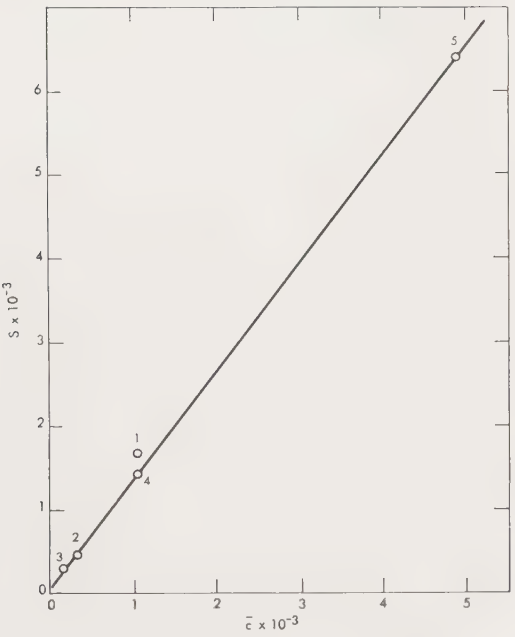

FIG. 6. Plot of standard deviation (S) of sardine egg counts per 10 m^2 of sea surface against their mean ($\bar{c}$) for grids 1 to 5.

their mean for the grids (Figure 7) shows that the proportionality between standard deviation and mean has been eliminated, and there is considerably more variability than previously. There is an indication that the relationship is now slightly inverse, which probably reflects the fact that high mean values are associated with fewer zeros. On a logarithmic scale, this represents a considerable restriction of the range of values since a variation from 1 to 10 is equivalent to a variation from 1,000 to 10,000.

Let y_{hij} be the logarithm of the count plus one for the hth grid, ith column and jth row. A linear model of the components contributing to the count is

$$y_{hij} = \mu + \alpha_h + \beta_i + \gamma_j + (\alpha\beta)_{hi} + (\alpha\gamma)_{hj} + (\beta\gamma)_{ij} + \varepsilon_{hij} \quad ,$$

where μ = over-all mean,
α_h = grid effect,
β_i = column effect,
γ_j = row effect,
$(\alpha\beta)_{hi}$, $(\alpha\gamma)_{hj}$, $(\beta\gamma)_{ij}$ = interaction terms
and ε_{hij} = error of individual observation.

TABLE 5. *Means, standard deviations, and totals of sardine eggs per 10 m^2 of sea surface for grids 1–5*

Grid	Mean	Standard deviation	Total
1	1,088	1,427	27,210
2	314	467	7,854
3	166	305	4,146
4	1,061	1,685	26,527
5	4,893	16,373	122,332

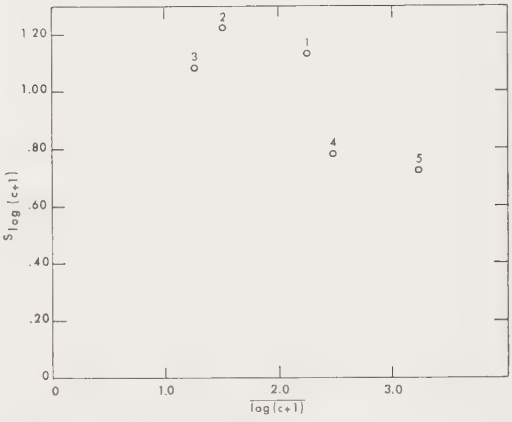

FIG. 7. Plot of standard deviation (S) of logs of sardine egg counts plus 1 per 10 m^2 of sea surface against their mean [$\overline{\log (c+1)}$] for grids 1 to 5.

As was indicated above, a row is considered to be an E-W section and a column to be a N-S section. An analysis of the variance of the logarithms of the counts plus one is presented in Table 6.

In order to test for the reality of any of the components, the deviations from the class means must be assumed to be normally distributed. The test for significance of a particular component of variance involves comparing it to the residual variation by means of the F test. Moderate departures from normality have been shown not to affect seriously the validity of the F test. However, large departures do affect the validity of the conclusions drawn from F tests. The effect of such non-normality is to raise the frequency of statistically significant results. In this data, since there are

TABLE 6. *Analysis of variance of logarithms of sardine eggs per 10 m² of sea surface plus one*

Source	Sum of squares	Degrees of freedom	Mean square
Between grid	62.07222	4	15.51806
Between row	0.37732	4	0.09433
Between column	68.13767	4	17.03442
Grid × row	9.03207	16	0.56450
Row × column	8.05534	16	0.50346
Grid × column	11.87116	16	0.74195
Residual	23.75211	64	0.37113
Total	183.29789	124	

characteristically a few very large observations, there may be some question as to the strict interpretation of the tests of hypotheses (Cochran 1947).

None of the interaction components are significantly different from zero when tested against the residual variation, thus indicating the additivity of the components on the logarithmic scale. The component of variance due to row position is not statistically different from the residual variation, indicating that the data can be considered homogenous in a direction parallel to the coast. Pooling the components that are not statistically different from zero, the new residual variance is 0.4577. The estimated variance between grid means is 0.6024, and the estimated variance between column means is 0.6631.

These variances apply to the logarithms, and it is desirable to refer these calculations back to the original scale of the egg counts. The relation between the variance of a log-normal variable σ^2_y and the coefficient of variation V_x of the original variable is given by Bagenal (1955).

$$\sigma^2_y = 0.434 \log_{10} (1 + V^2_x) , \qquad (12)$$

where V^2_x is the relative variance of x. This equation may be solved for the coefficient of variation

$$V_x = \left(\text{antilog}_{10} \frac{\sigma^2_y}{0.434} - 1 \right)^{1/2} . \qquad (13)$$

Using (13) the coefficients of variation of grid and column means and the residual are: $V_g = 4.8$; $V_c = 5.7$; and $V_o = 3.2$. The coefficient of variation expresses the standard deviation as a multiple of the mean. Thus for any mean level, the standard deviation can be estimated by multiplying the coefficient of variation by the mean.

The residual variation can be compared with the haul to haul variance estimated by Silliman (1946). Silliman concluded that the variance of the logarithm of a single count from a single haul is 0.031. This value corresponds to a coefficient of variation of 0.42, which is 13% of the coefficient of residual variation estimated from the grid. The residual error estimated from the grid observations includes all of the variability not accounted for by the variation in mean between grid and N-S sections (columns). Therefore, the estimated residual error cannot be interpreted as measuring the error of a single haul. The estimates V_g and C_c will be accepted as valid estimates of the variation introduced from these sources. These variances are many times the estimated haul to haul variability of Silliman and indicate that contributions to the variance from his haul to haul variability over one mile are quite small relative to contributions observed here from hauls made four miles apart and hauls made a day apart at the same station. The importance of these sources of variation must be recognized when a large area of the sea surface is represented by a single plankton collection taken from this area.

Using these estimates of variation it is interesting to calculate the variance of two types of estimates of abundance. To estimate the population over the five day period, we can sum the counts per unit area of sea surface of the 125 stations weighted by an appropriate constant area factor w_o .

$$C_{\ldots} = w_o \sum_h \sum_i \sum_j c_{hij}$$

As an alternative we can let a single station represent the total area and period of five days.

$$C^* = 125\ w_o c_{hij}$$

Since in the first case we are summing over the time and area strata, no account need

to be taken of the variance between these strata. We will assume that the variation for the first case is fully described by the coefficient of variation between hauls (V_s) of Silliman (1946). We are thus assuming a model in which the variation between one-half mile hauls is taken to be representative of an area 16 square miles, and also differences between such areas and the grids are described by the estimated components of variance for columns and grids. Since the counts tend to be distributed in a steady gradient, it is unlikely that the data are homogenous within a 16 square mile area. Counts taken from one side of the standard area compared to counts taken on the other side can be expected to differ more than counts from contiguous one-half mile oblique hauls. The use of Silliman's figure for haul to haul variation will tend to emphasize the importance of between strata variation and thus exaggerate the relative error of the estimate C*. In actuality, the proper value for the residual variation should be between Silliman's figure and the residual variation estimated from the grid.

The ratio of the variances of $C_{...}$ and C* is

$$\frac{S^2_{C...}}{S^2_{C*}} = \frac{V_s^2}{125\ (V_s^2 + V_c^2 + V_g^2)} = 0.00019$$

The ratio of the standard deviations is accordingly 0.0138. The variance of a population estimate based on a single sample is

$$S^2_{C*} = (125)^2\ w_o^2\ (V_s^2 + V_c^2 + V_g^2)\ m^2\ .$$

The relative variance of C* is 970.2 and the corresponding coefficient of variation is 31.1. This coefficient of variation is very large and indicates that an estimate of population abundance from a single sample is such that the standard deviation will be approximately 30 times the count. Silliman (1946) showed that a logarithmic transformation of his egg counts yielded a bell-shaped distribution which could be considered normal. Since the present grid observations are highly stratified, it is impossible to verify the normalizing influence of the log transformation for this data. Even though the effect of such a transformation cannot be established, it is instructive to assume that the counts are normally distributed on a logarithmic scale. The estimated coefficient of variation $V_{C*} = 31.1$ then implies that we can be practically certain that the relative error in the sample is not greater than twice the coefficient of variation, or 62.2 (Hansen, *et al.*, 1953, Vol. I, p. 125).

The relative variance of a sum of counts drawn from such a distribution will be $\frac{V^2_{C*}}{n}$, where V^2_{C*} is the relative variance of a single sample and n is the number of counts entering into the sum. The relative variation of the sum will accordingly be less than that of the individual samples. For example, the relative variance of a sum of 40 counts from this population would be 24.3, and the coefficient of variation 4.9. This implies that an estimate of the total number of eggs in such an area from 40 samplings will most likely have a relative error less than 10.

DISCUSSION

Recently some attention has been devoted to the design of surveys to estimate fish populations whose distribution is not random. The implications of a contagious distribution of fish for the design of trawl surveys to estimate population size have been discussed by Taylor (1953). Since the requirements of a survey to estimate the abundance of a planktonic population are somewhat different, it is important to examine the applicability of his conclusions to plankton sampling.

Data collected on surveys of the waters off southern California were shown to be consistent with the hypothesis of an underlying negative binomial distribution of sardine eggs. The means were high, 243-976 eggs per 10 m^2 of sea surface, and the estimated values of the contagion coefficient, K, ranged from 0.14 to 0.76. The integration over time will not be considered here, and each station will be taken to represent an equal area. Let the total area to which the count is applied be A, and the size of the sampling unit be a. Therefore there are $A/a = P$ sampling units in the area of sur-

vey. An estimate C of the total number of organisms in the area A will be $C = (A/a)c$, with variance $\sigma^2(C) = (A/a)^2 \sigma^2(c)$. If the counts are distributed according to the negative binomial probability density with mean m and contagion coefficient K,

$$\sigma_C^2 = \left(\frac{A}{a}\right)^2 \left(m + \frac{m^2}{K}\right) .$$

The value of *a* can be chosen by the sampler and will determine the value of the sample mean. Since the value of K is determined by the distribution of the eggs, its value will be independent of the size of the sampling unit. As the size of the sampling unit increases (decreases) the mean of the *samples* will increase (decrease). I will assume a proportionality between sample mean and size of sampling unit.

Taylor (1953) considered the problem of how to choose the number of tows and the size of the sampling unit to provide the best estimate of the population on the assumption that the total fraction of the survey area sampled remains a constant. This condition implies that if the size of the sampling unit is increased from a_1 to a_2 then the number of samples is decreased from n to $\frac{a_1}{a_2}$ n. Under this condition Taylor shows that a smaller sampling unit is more efficient than a larger sampling unit if the population sampled is distributed according to a negative binomial distribution. He defines the relative efficiency of two estimates as the relative amount of sampling required to give estimates of equal variance.

The requirements of certain kinds of plankton survey programs are often not expressed in this model. The number and distribution of samples are often fixed by the known approximate distribution in space and time of the organism and the number of ship hours available for the survey. In the case of collecting plankton with a conventional plankton net in an oblique or vertical tow, for example, a doubling of the size of the mouth of a plankton net used in sampling the surface layers will not materially affect the proportion of time spent in operation of the nets relative to the total time of the cruise. Under the assumption of independence of the number of samples and the size of the sampling unit, I will consider the effect of changing the size of the sampling unit on the precision of the estimate of abundance at a single station. The conclusions will also apply to a summation of the counts.

The effect of changing the size of the sampling unit on the precision of the estimation can be seen by considering the relative precision of two estimates C_1 and C_2 based on different size sampling units a_1 and a_2 . These estimates are both unbiased but will be seen to possess different precisions. The precision of an estimate is defined as the reciprocal of the variance of the estimate. The relative precision of two estimates C_1 and C_2 will be

$$\text{R.P.} = \frac{\sigma^2(C_2)}{\sigma^2(C_1)} .$$

If the ratio is greater than 1 then C_1 is more precise than C_2.

$$\text{R.P.} = \frac{\left(\frac{A}{a_2}\right)^2 \left(m_2 + \frac{m_2^2}{K}\right)}{\left(\frac{A}{a_1}\right)^2 \left(m_1 + \frac{m_1^2}{K}\right)}$$

$$= \frac{\frac{a_1}{a_2} m_1 + \frac{m_1^2}{K}}{m_1 + \frac{m_1^2}{K}}$$

If $a_1 > a_2$ then R.P.>1. Thus, if C_1 is based on a larger sampling unit than C_2 it will be more precise and the reverse is true if C_1 is based on a smaller sampling unit. This difference arises from a difference in the Poisson component of the variance and leaves the second term in the expression unchanged. For many sampling problems, *e.g.* the estimation of sardine egg populations, the first term is quite small relative to the second term, and the estimates for practical purposes may be considered equally pre-

cise. I will use the extreme values of m and K estimated from the egg data and see what effect on the precision results from a change of the size of the sampling unit of 2. The conditions for maximum effect on precision of a change in size of sampling unit exist when the first term is largest relative to the second, or when the mean is low and K is high. For a mean of 250 eggs per 10 m^2 of sea surface and a $K = 0.75$, the relative precision of C_1 to C_2 is 1.003. For practical purposes in the sampling of sardine eggs in a given area, it is clear that the precision of the estimate is independent of the size of the sampling unit. It would only have an effect for extremely low counts or distributions with a very low degree of contagion, and these conditions appear to be uncommon in the distribution of sardine eggs in the sea.

Ahlstrom (1954) has shown that real differences exist in the density of sardine eggs between subregions of the total area of sardine spawning. Sampling theory shows the desirability of dividing the total area up into subareas in which the eggs are more or less distributed uniformly and taking a random sample within the subareas (Tanaka 1955). Provided that the variation in subarea means is large relative to variation within each subarea, the reduction in variance of the estimate will be considerable. The theory of stratified sampling states that the optimum allocation of randomly chosen samples per subarea occurs if the number of samples is proportional to the standard deviation within each stratum. In the case of sardine eggs, the standard deviation is roughly proportional to the mean in the range of means usually encountered. Thus, a stratified sample estimate should be designed so that the number of stations per stratum is proportional to the mean density within that stratum.

The application of such a sampling scheme to the estimation of the abundance of sardine eggs, although appealing, presents some difficult problems. The differences between subregions are not consistent from one year to the next. Often they are not even consistent from one month to the next. There does not seem to be much evidence of preferred geographic areas. The distribution of spawning is most likely determined by the interaction of the biology of the species and the oceanographic conditions. As the relationships between the distribution of spawning fish and oceanographic conditions are not yet well understood, a stratified sample design to estimate the population cannot be proposed with any confidence. One possibility might be to take additional tows adjacent to a haul that yields a high density of eggs. The return for such a scheme would be the reduction of the variance of the estimate of the eggs in the subarea of high density by a factor of $\frac{1}{n}$, where n is the number of tows per subarea.

The possibility of integrating samples horizontally has been investigated by Ahlstrom, *et al.* (1958). The high speed sampler used in this study integrates a large number of very small samples over the path of the instrument. The conclusion reached by these authors was that sampling of sardine eggs with high speed samplers that sampled a horizontal strip of water over 10 miles at several depths was much more effective for population estimation than sampling with oblique hauls spaced at 40 mile intervals. By this method it is possible to sample a number of different clumps of eggs, and thus reduce the contribution of between clump variability of mean to the variability of areal estimates. This type of sampling is advantageous when the horizontal distribution of the organisms poses a more serious obstacle to taking a representative sample than the depth stratification. Such a method, if consistent with the other objectives of the survey, holds real promise in increasing the precision of population estimates of contagiously distributed plankton.

CONCLUSIONS

In this paper, the reliability of estimates of sardine egg abundance is estimated from several points of view. Since these conclusions apply only to the sets of data examined

here, no firm generalization can be made about the variability of egg estimates. An examination of other egg collections would no doubt yield different results. The analysis of these egg distributions does indicate the order of variability encountered, and serves as a more realistic standard with which to judge the reliability of sardine egg estimates than presently exists in the literature.

The analysis of the spawning surveys of 1941 showed that the data could be considered in months of significant spawning to be drawn from a population of eggs distributed in nature according to the negative binomial distribution. By a suitable transformation of this data, a transformed variable y is obtained whose distribution could be presumed to be approximately normal. The 95% confidence limits for the estimate of the total varied in relative terms from 1.4 to 2.5. Thus, on the average these cruise estimates are subject to a sampling error such that the true value may be one-half or double the estimated value. Although the summation of cruise totals to give a seasonal estimate appears to be more reliable, a large component of variance due to variability of egg counts at any station over the time interval between occupancies is not included in the estimate of variability of the seasonal estimate. The grid studies showed that variability in time is almost as large as variability in space. Thus, the seasonal estimate cannot be considered to be more precise than any of the cruise estimates, and may be considerably less precise.

This work extends the treatment of the same data by Sette and Ahlstrom (1948). The variability estimated here arises from various sources not dealt with in detail by these authors. Their variability was based on the haul to haul variability estimated by Silliman (1946) from paired hauls taken one-half mile apart. This standard of variability underestimates the true variability since tows are taken to represent a 400 square mile area and counts taken randomly within this area at distances greater than one-half mile can be expected to vary more than contiguous one-half mile tows. Also, the component of variance due to changes between occupancies is not explicitly recognized. For these reasons it is my belief that Sette and Ahlstrom (1948) have understated the errors in these sardine egg surveys.

The estimates of variability from the grid survey of a 400 square mile area demonstrate clearly the importance of real spatial and temporal changes in the distribution. The mean level observed depends very markedly on the time of arrival of the research vessel at a particular station. For instance, the order of occupancy and position from which a survey vessel starts may strongly influence the number and distribution of eggs collected. In a like manner, the actual position of occupancy within a standard 400 square mile area influences the size of the collection of eggs. The relative variation of an estimate of the grid total from a single station was of the order of 30 at the one standard deviation level. Since this constitutes only a description of the distribution in one 400 square mile area, it is not possible to generalize on the variation to be found in other such areas. Forty repeated samplings from such an area would yield an order of variation of slightly less than five at the one standard deviation level. This variation is significantly greater than the variation observed in the analysis of the spawning surveys of 1941. Since these surveys deal with phenomena of such widely different scale, the results are not strictly comparable. The distribution of eggs within this small area, approximately the size of the basic sampling area of the 1940–1941 surveys, does emphasize the order of magnitude of the changes in sardine egg concentration that take place over time periods of one day, and distances of four miles.

The implications of the observations presented here for the design of egg surveys are discussed. It is concluded that the precision of the estimation is virtually independent of the size of the sampling unit if the size of the sampling unit is independent of the number of observations. The returns for designing a survey in which the number of samples per unit area is proportional to the

mean density within a subarea is pointed out. The possibility of reducing the variances of the estimates observed here by integrating samples horizontally, instead of taking vertically integrated samples, is discussed (Ahlstrom, *et al.* 1958).

REFERENCES

Ahlstrom, Elbert H. 1943. Studies on the Pacific pilchard or sardine (*Sardinops caerulea*) 4. Influence of temperature on the rate of development of pilchard eggs in nature. U.S. Dept. Int., Fish and Wildl. Service, Spec. Sci. Rept., No. 23. 26 pp.

———. 1948. A record of pilchard eggs and larvae collected during surveys made in 1939 to 1941. U.S. Dept. Int., Fish and Wildl. Service, Spec. Sci. Rept., No. 54. 76 pp.

———. 1954. Distribution and abundance of egg and larval populations of the Pacific sardine (*Sardinops caerulea*). U.S. Dept. Int., Fish and Wildl. Service, Fish. Bull., **56**(93): 83–140.

———, J. D. Isaacs, J. R. Thrailkill, and L. W. Kidd. 1958. High speed plankton sampler. U.S. Dept. Int., Fish and Wildl. Service, Fish. Bull., **58**(132): 187–214.

———. 1959. Vertical distribution of pelagic fish eggs and larvae off California and Baja California. U.S. Dept. Int., Fish and Wildl. Service, Fish. Bull., **60**(161): 107–146.

Anscombe, F. J. 1948. The transformation of Poisson, binomial and negative binomial data. Biometrika, **35**: 246–254.

———. 1949. The statistical analysis of insect counts based on the negative binomial distribution. Biometrics, **5**: 165–173.

Bagenal, M. 1955. A note on the relations of certain parameters following a logarithmic transformation. J. Mar. Biol. Assoc. U. K., **34**: 289–296.

Barnes, H. and S. M. Marshall. 1951. On the variability of replicate plankton samples and some applications of contagious "series" to the statistical distribution of catches over restricted periods. J. Mar. Biol. Assoc. U. K., **30**: 233–263.

Bartlett, M. S. 1947. The use of transformations. Biometrics, **3**: 39–52.

Bliss, C. I. 1953. Fitting the negative binomial distribution to biological data. Biometrics, **9**: 176–196.

Clark, F. N. and J. C. Marr. 1955. Population dynamics of the Pacific sardine. *From:* Progress Report, 1 July 1953–31 March 1955, State of California, Dept. of Fish and Game, Mar. Res. Comm., pp. 13–48.

Cochran, W. G. 1947. Some consequences when the assumptions for the analysis of variance are not satisfied. Biometrics, **3**: 22–38.

Davis, H. T. 1933. Tables of the higher mathematical functions. Vol. 2. Principia Press, Inc., Bloomington. pp. 27–58.

Fisher, R. A. 1953. Note on the efficient fitting of the negative binomial. Biometrics, **9**: 197–199.

Gulland, J. A. 1956. A note on the statistical distribution of trawl catches. Cons. Int. Explor. Mer. Rapp. et Proc.–Verb des Reunions, **140**: 28–29.

Hansen, M. H., W. N. Hurwitz and W. G. Madow. 1953. Sample survey methods and theory. Vol. I: Methods and applications, John Wiley and Sons, Inc., New York. 638 pp.

———. 1953. Sample survey methods and theory. Vol. II: Theory. John Wiley and Sons, Inc., New York. 332 pp.

Holmes, R. W. and T. M. Widrig. 1956. The enumeration and collection of marine phytoplankton. J. Cons. Int. Explor. Mer, **22**: 21–32.

McEwen, G. F., M. W. Johnson, and T. R. Folsom. 1954. A statistical analysis of the Folsom Plankton Splitter, based on test observations. Archiv für Meteorologie, Geophysik, und Bioklimatologie, Ser. A. **7**: 502–527.

Sette, O. E. 1943. Structure of a research program to determine how fishing affects the resource. U.S. Dept. Int., Fish and Wildl. Service, Spec. Sci. Rept., No. 19. 27 pp.

——— and E. H. Alstrom. 1948. Estimation of abundance of the eggs of the Pacific pilchard (*Sardinops caerulea*) off southern California during 1940 and 1941. J. Mar. Res., **7**: 511–542.

Silliman, R. P. 1946. A study of variability in plankton tow-net catches of Pacific pilchard (*Sardinops caerulea*) eggs. J. Mar. Res., **6**: 74–83.

Tanaka, S. 1955. Estimation of the abundance of the eggs of fish such as sardine. I. Method of estimation by the vertical haul of the plankton net. Bull. Jap. Soc. Scien. Fisheries, **21**: 382–389.

Taylor, C. C. 1953. Nature of variability in trawl catches. U.S. Dept. Int., Fish and Wildl. Service, Fish. Bull., **54**(83): 145–166.

Winsor, C. P. and G. L. Clarke. 1940. A statistical study of variation in the catch of plankton nets. J. Mar. Res., **3**: 1–34.

——— and L. A. Walford. 1936. Sampling variations in the use of plankton nets. Jour. Cons. Int. Explor. Mer, **2**: 190–204.

SOME LIME-INDUCED CHANGES IN LAKE METABOLISM

Raymond G. Stross[1] *and A. D. Hasler*

Department of Zoology, Hydrobiology Laboratory, University of Wisconsin, Madison

INTRODUCTION

Application of hydrated lime to bog and other dystrophic lakes is thought to stimulate production at several trophic levels. It enhances the medium for plant growth by increasing: the rate of remineralization of plant nutrients (Hasler and Einsele 1948); the depth of the euphotic zone (Hasler *et al.* 1951) and the supply of available carbon dioxide (Hasler *et al.* 1951, Waters 1957, Waters and Ball 1957). In addition, phosphate should be conserved in the trophogenic layer as a result of the removal of iron from suspension. The consequences of phosphate removal by ferric-iron complexes are discussed by Einsele (1938), Ohle (1937a) and Mortimer (1941, 1942).

Lakes treated with lime have not responded uniformly owing, perhaps, to the differences in the nature of the lakes and method of treatment. Hasler *et al.* (1951) and Johnson and Hasler (1954) have enlarged the euphotic zone in several instances although the latter workers and Waters (1957) were unable to clear some lakes. Large applications of hydrated lime result in plankton blooms and probably are induced by the momentary release of phosphorus (Waters). But whether the increase in plant production is transferred to all trophic levels is questionable. Johnson and Hasler report no increase in production of rainbow trout following an increase in standing crop of plankton. However, Waters and Ball show an increase in plankton and growth rate of yellow perch.

This paper considers some of the direct effects of lime on lake metabolism. A lake previously treated with apparent success was used to test the result of treatment on lake dystrophy and the distribution of iron and phosphorus. Because of its possible significance in achieving and maintaining desired results, a method of application was studied in two other lakes by measuring the efficiency of solution of lime and the degree of retention of the dissolved products. The effect of lime-treatment on the production of planktonic crustacea will be reported elsewhere.

Appreciation is extended to the Guido Rahr Foundation for a research grant, to the University of Notre Dame and Rainbo Lodge for facilities, and also to Drs. J. C. Neess, W. R. Schmitz and W. J. Wisby for their helpful suggestions.

THE LAKES

Three lakes on the Wisconsin-Michigan border near Land O' Lakes, Wisconsin were selected for the studies. All are slightly acid and are stained with organic colloids. Bog plants grow on a portion of the shorelines, and peat-like organic materials are deposited on the bottoms, except in some of the shallow areas where wave action has exposed the underlying sand. The restricted drainage basin surrounding each lake has not permitted permanent tributaries to develop. George and Corrine lakes are slightly elongated; whereas, Peter-Paul Lake consists of two concentric basins which were connected by a narrow, shallow canal, in hourglass fashion. This lake was separated in 1951 by an earthen dam (Johnson and Hasler 1954). Peter Lake (treated portion) received treatment with hydrated lime beginning in 1951 and continuing until 1954. Paul Lake (untreated portion) was preserved for use as a natural reference. The area, volume, and maximum depth of each lake are given in Table 1.

APPARATUS AND PROCEDURE

To provide maximum homogeneity and uniform distribution, the lime was slurried and applied with the aid of a high speed centrifugal pump. The pump and lime receptacle were attached to a large boat

[1] Present address: Department of Zoology, University of Maryland, College Park, Maryland.

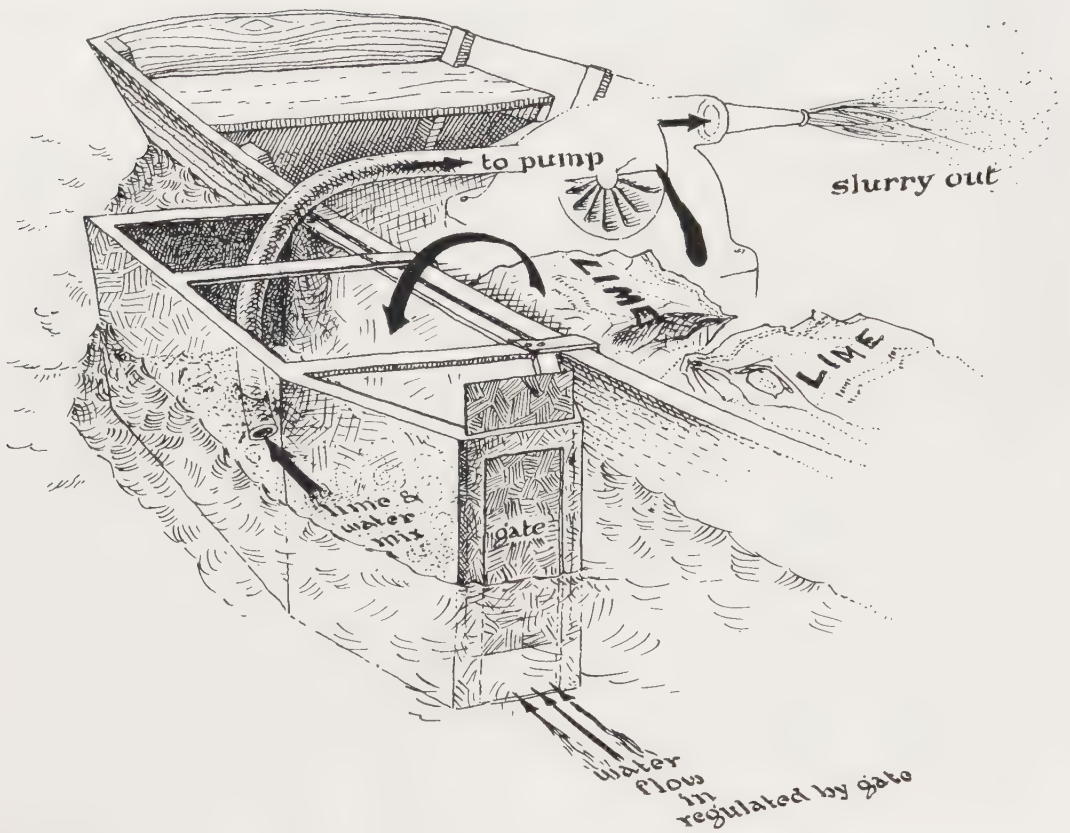

FIG. 1. Apparatus used for application of lime.

capable of transporting the apparatus and a supply of lime about the lake (Fig. 1). When the water level was adjusted correctly, the addition of lime to the receptacle was an almost continuous operation.

Temperature and light measurements were obtained with Whitney instruments from a station in the center of each lake as were samples of water for chemical analyses. Water samples were filtered within a few hours through fine-porosity-glass filters to separate "soluble" and "particulate" fractions.

Calcium, magnesium, sodium and potassium were measured by flame photometry in a Beckman D.U. spectophotometer after the water sample had been concentrated to ⅕ of its initial volume. Total phosphorus was measured by the molybdate-blue procedure (Truog and Meyer 1929) in samples concentrated to 1/10 of their initial volume. Total iron was determined by an orthophenanthroline technique as used by Neess (personal communication) which consisted of complete oxidation to ferric iron followed by reduction with hydroxylamine hydro-

TABLE 1. *Morphometric features of the lakes*

Lake	Area (Acres)	Area (ha)	Max. Depth (m)	Volume (m^3)	Axis
George	42.8	17.3	14.0	778,000	WSW–ENE
Corrine	36.1	14.6	5.2	350,000	N–S
Peter (treated)	6.0	2.4	19.0	436,000	neutral
Paul (untreated)	3.0	1.2	12.0	134,000	neutral

chloride. Bicarbonate was calculated from the milliequivalents of acid required to bring a sample to a methyl-orange endpoint and was expressed as bicarbonate; calculations were made only when the pH of the sample indicated carbonate to be insignificant (Hutchinson 1957). Measurements of pH were made with a Beckman G pH meter.

Hydrated lime with an average content of 30.5% calcium and 17.0% magnesium, and an hydroxide equivalent of 96.5% was used throughout the study. These values were used to calculate theoretical amounts in each application. Only calcium and magnesium were considered in calculating efficiencies of solution, and their hydroxides were assumed to dissolve in equal molar quantities. Other cations in the lime were assumed to compensate for introduced "non-carbonate" salts.

RESULTS

Efficient solution of lime

Two treatments with hydrated lime were made in George and Corrine lakes in the summers of 1956 and 1957, respectively. Each treatment added a calculated 5.8 mg/L of lime to George Lake and 12.9 mg/L to Corrine Lake. A difference in the time-interval and quantity of each application provided an indication of the effect of available carbon dioxide on the efficiency of solution. In George Lake a six-week period separated the two additions during which time the pH of the epilimnion returned from a post-treatment value of 9.4 to 7.2. The second application to Corrine Lake followed five days after the first and at a time when most of the lake was still at a post-treatment pH of 9.7.

The two applications to George Lake increased bicarbonate by 85 and 101% of the theoretical value. In Corrine Lake the increase was only 54%. Because the actual change was comparable in the two lakes, the lower efficiency obtained in Corrine Lake probably was the result of larger application of lime and smaller interval between treatment. Thus, efficiency of solution seemed to be dependent mainly on the amount of carbon dioxide immediately available for reaction with the hydroxides.

In George Lake the distribution of lime, as indicated by change in bicarbonate, was uniform with the exception of a region in the lower part of the thermocline (Fig. 2). Because the stratum was well beneath the zone where precipitation could occur as a result of photosynthesis, it is suggested that one size-group of particles dissolved in the epilimnion and another sank into the hypolimnion before dissolving. On the other hand, lime was distributed exclusive of the hypolimnion in Corrine Lake. Apparently, most of the undissolved lime was lost to the littoral region, and that which dissolved in the narrow stratum at the bottom was subsequently adsorbed by the sediments. Evidence for the entrance of lime into this stratum is presented below.

Calcium and magnesium

Direct determinations of calcium and magnesium in Corrine Lake permitted a measure of their retention and distribution. Table 2 gives the initial concentration, the amount added and the fraction of each which remained in the water column. The subsequent decline in the amount of calcium suggested that all may not have been in solution, although the formation of bicarbonate was sufficiently large to account for the maximum listed values of calcium and magnesium. Conceivably, calcium dissolved to the same extent after each application, as indicated by the amount present one week after application, but was subsequently lost from solution.

Magnesium salts were less soluble at the time of the second application and, presumably, less soluble than were the calcium salts. This may be expected since a water pH of 9.7 indicated an absence of free carbon dioxide, in which case calcium hydroxide should have been the more soluble. The net increase in calcium and magnesium, as measured four weeks after the second addition, was 38% of the theoretical value with magnesium comprising ⅔ of the total.

The smaller efficiency for solution of cations can be accounted for only if calcium

TABLE 2. *Amount of calcium and magnesium in solution and retained following application of hydrated lime to Corrine Lake*

Day	Calcium			Magnesium		
	Added (Kg)	Retained (Kg)	(%)	Added (Kg)	Retained (Kg)	(%)
0	1383	—	—	771	—	—
7	—	615	44	—	546	71
7	1383	—	—	771	—	—
9	—	857*	62	—	340*	44
14	—	580	42	—	300	39
34	—	139	10	—	345	44
Net increase		754	27		891	58

* Increase over and above that measured on day 7.

was adsorbed and precipitated by colloids in suspension and on the bottom (Ohle 1937b, 1955). This was indicated because of its continually declining concentration. Calcium also failed to accumulate in the 1-meter stratum near the bottom while magnesium increased sevenfold. Assuming only calcium was involved, approximately 16% was adsorbed after it dissolved.

The adsorption of calcium was also indicated in Peter-Paul Lake (Table 3). Despite the probability of an unrecorded application, the assumption regarding differential adsorption should remain the same. To satisfy the excess bicarbonate, which existed after several years, would require 24% more calcium than existed in solution. The apparently efficient solution of lime in this lake, although applied as a powder, was thought to be due to modest additions and depth of the lake.

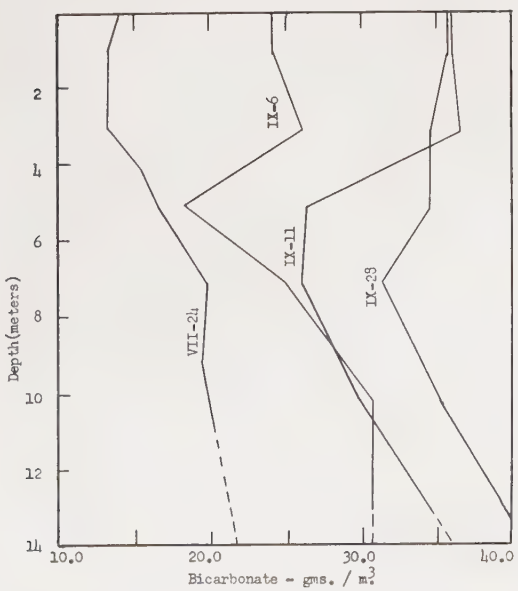

FIG. 2. Vertical distribution of bicarbonate in George Lake. Applications of hydrated lime were made on July 24 and September 6, 1956.

Light penetration

The depth of the euphotic zone increased by 22% in George and 40% in Corrine Lake following lime treatment. In George Lake clearing followed only the first application; the second, made near the time of the fall overturn, merely counteracted the normal resuspension of materials which usually narrowed the euphotic zone during the overturn of lakes in the area. Johnson and Hasler (1954) reported a 60% increase in the treated portion of Peter-Paul Lake. Readings taken two years later showed a total increase of 160%, a change from 2.7 to 7.0 m. The significance of the additional change was reduced by a proportional in-

TABLE 3. *Amount of calcium and magnesium retained in solution and the formation of bicarbonate from hydrated lime added over a 3-year period to the treated portion of Peter-Paul Lake. Measurements were made five years after the beginning of treatment.*

	Addition (mg/L)	Retained in solution (mg/L)	Efficiency (%)
Ca	4.35	2.17	50
Mg	2.47	2.38	96
HCO_3	25.6	20.8	81

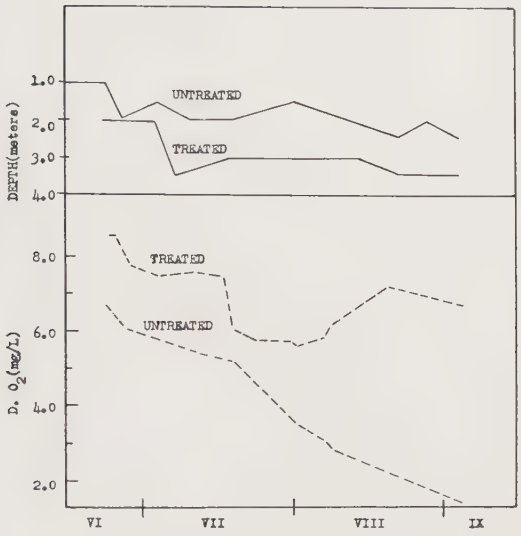

FIG. 3. Depth of the epilimnion in treated and untreated portion of Peter-Paul Lake and the changes in concentration of dissolved oxygen in each portion during the summer of 1956.

crease in depth of the euphotic zone in the untreated portion.

Lake dystrophy

Hasler *et al.* (1951) attributed the slower decline in concentration of dissolved oxygen in the thermocline of lime-treated lakes to the dual action of increased photosynthesis and decreased oxygen consumption, which result from deeper penetration of light and removal of organic suspensoids, respectively. To determine the relative importance of the two, a series of 8 light and dark bottle measurements were carried out at equal light intensities in the thermoclines of the treated and untreated portion. Because the thermoclines are located at different depths (Fig. 3) as a result of deeper light penetration in the treated portion (Stross 1958), equivalent light intensities occurred at 5.0 m in the treated and 3.0 m in the untreated portion. Light values at these depths ranged from 3.0 to 6.0% of surface radiation during the test period. The bottles were incubated for 8 hr. The change in amount of dissolved oxygen was measured by the unmodified Winkler technique.

The decline in concentration of dissolved oxygen in the upper thermocline of the untreated portion was due mainly to a high rate of respiration. A faster rate of consumption in this portion was readily apparent (Fig. 3). Presumably, no excess oxygen was produced during the daylight period because both production and consumption of oxygen were 28.5 μg/L/hr. In the treated portion, however, the values were 38.7 and 12.2 μg/L/hr., respectively, an oxygen demand of only 32% of that produced.

Iron and phosphorus

Peter-Paul Lake was selected for evaluating the effect of hydrated lime on the distribution of iron and phosphorus. Because application was stopped when this study began, the conditions present in the lake two years later were thought to represent a persistent influence of treatment on the two elements. Existing concentrations and distributions in the untreated portion at the time of measurement were considered representative of those in the treated portion before the initial application.

The seasonal distribution of iron conformed with that summarized by Hutchinson (1957). Because the lakes circulated completely in autumn only, iron was homogeneously distributed at that time, but only for a brief period which preceded a rapid loss from suspension (Fig. 4). At overturn the concentration was 0.20 mg/L in the treated and 0.65 mg/L in the untreated portion. The ability of lime to remove iron permanently from circulation was therefore suggested; presumably, lime eliminated 70% of the iron.

Particulate iron comprised 30% of the total amount in each lake. There was, however, considerable variation, particularly at the time of overturn (Fig. 4). A greater proportion became particulate at the spring overturn, but because circulation was incomplete, the precipitating iron redissolved in the anaerobic stratum near the bottom. The greater accumulation occurred in the hypolimnion of the untreated lake, being 4.3 mg/L as compared to 1.8 mg/L in the treated portion.

Because ferric iron precipitates phosphorus from the euphotic zone of lakes, it

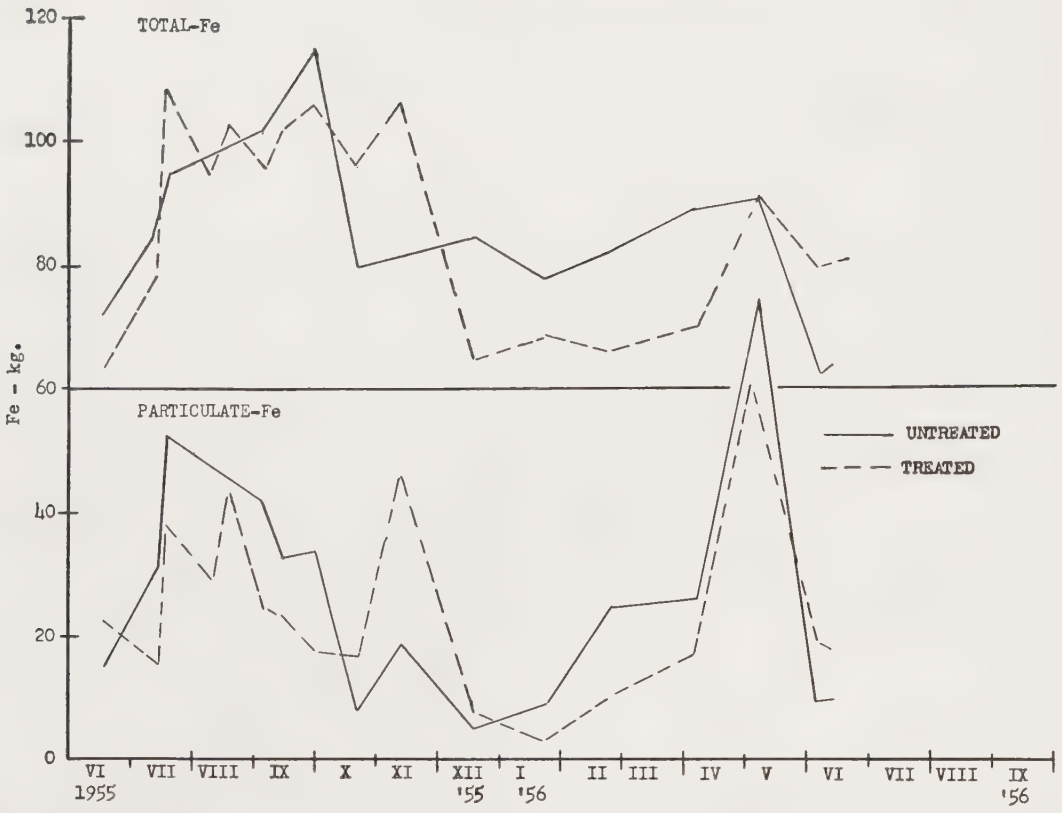

FIG. 4. Concentrations of total and non-filterable iron in the two portions of Peter-Paul Lake. The volume of the untreated portion is 30% of the other.

was of interest to determine if the distribution of phosphorus was affected by the partial removal of iron. A set of six measurements taken during the winter-to-spring period suggested the partial removal did not inhibit normal precipitation (Fig. 5). Instead, loss of phosphorus in the treated portion seemed to occur more rapidly and resulted in an average concentration of 22.0 μg/L as compared to 28.0 μg/L in the untreated part of Peter-Paul Lake. A nonbiological precipitation was thought to be the major cause for the loss because the plankton was extremely sparse during the period. The major difference in distribution was the appearance of a large concentration of phosphorus in the surface waters of the treated portion. A maximum was first noted in early February at a depth of one meter. This layer moved downward slowly and arrived at the bottom in late April. There seemed to be, however, no apparent correlation with the downward movement of iron.

DISCUSSION AND CONCLUSIONS

Complete solution of hydrated lime may be obtained if the addition does not greatly exceed the reservoir of free carbon dioxide. The importance of carbon dioxide is illustrated by the comparable amounts of lime which dissolved in George and Corrine lakes, although dosages were 6.0 and about 26.0 mg/L respectively. Although dosage and efficiency were not exactly proportional, the low efficiencies obtained by other investigators probably occurred because of the extremely high concentrations applied by them (Waters 1957; Waters and Ball 1957).

Odum (1956) suggests the degree of dystrophy is equivalent to the extent that

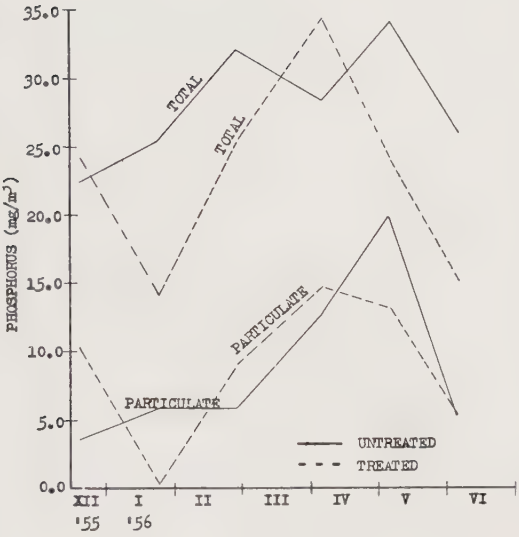

FIG. 5. Total and particulate phosphorus in the two portions of Peter-Paul Lake.

respiration exceeds photosynthesis. Because lime does not remove all organic colloids equally well, values of light penetration alone after treatment, may not measure adequately the change in degree of dystrophy. That the nature of the colloids is peculiar to lake-type is supported circumstantially by the fact that lakes which fail to clear following lime treatment are similar in being completely surrounded by a bog mat. Such lakes will respond, however. One such lake was maintained in a surprisingly clear state by temporary acidification with sulfuric acid to pH 4.0 (Zicker 1955). This variability of response suggests a difference in physical properties of the colloids. It may also imply variable stability to chemical oxidation. Conceivably, lime may precipitate efficiently only the more unstable colloids, in which case the rate of oxygen consumption may be decreased without changing the clarity of the water to any measurable extent. That some of the limnohumic acids are stable to oxidation was demonstrated by Shapiro (1957).

The normal precipitation of phosphorus by iron seemed unaffected by the addition of lime to Peter-Paul Lake. This occurred despite removal of nearly ¾ of the iron from suspension. At the autumnal overturn there was an iron to phosphorus ratio of approximately 3:1 which, according to Mortimer (1941), would seem to be sufficiently small to permit greater retention of phosphorus. However, a removal of colloids, which was indicated by the increase in clarity of the water, may have partially eliminated a mechanism for keeping phosphorus in suspension (Ohle 1937b) and thus, may account for the efficiency with which the iron remaining can act as a precipitating agent for phosphorus.

In a recent paper MacPherson *et al.* (1958) demonstrate that the release and uptake of phosphorus from reconstituted lake muds is dependent upon pH. Alkalization enhances release. A similar result was observed after the addition of lime to lakes by Waters (1957) and may also be inferred in other studies from the blooms of phytoplankton which followed the addition. Mimimal releases of phosphorus occur at a pH range of 5.5–6.5. Because pH in the three lakes of this study returned to 6.0–7.2, depending on depth, within a few weeks after treatment, the initial rate of flow from the muds probably was greatly slowed. This of course does not imply a lack of change in availability in subsequent years. In Peter-Paul Lake for example it is possible that as a result of treatment with lime a larger proportion of phosphorus was precipitated on the littoral sediments. Treatment may or may not have facilitated the release of phosphorus upon the necessary appearance of a reduced microzone at the mud-water interface.

REFERENCES

EINSELE, W. 1938. Über chemische und kolloid chemische Vorgänge in Eisen-Phosphat-Systemen unter limnochemischen und limnogeologischen Gesichtspunkten. Arch. Hydrobiol., **33**: 361–387.

HASLER, A. D. AND W. EINSELE. 1948. Fertilization for increasing productivity of natural inland waters. Trans. N. Amer. Wildl. Conf., **13**: 527–555.

———, O. M. BRYNILDSON AND W. T. HELM. 1951. Improving conditions for fish in brown-water bog lakes by alkalization. Jour. Wildl. Mgmt., **15**: 347–352.

HUTCHINSON, G. E. 1957. A treatise on limnology. Vol. 1. John Wiley & Sons, Inc. N.Y. xiv + 1015 pp.

JOHNSON, W. E. AND A. D. HASLER. 1954. Rainbow trout production in dystrophic lakes. Jour. Wildl. Mgmt., **18**: 113–134.

MACPHERSON, L. B., N. R. SINCLAIR AND F. R. HAYES. 1958. Lake water and sediment. III. The effect of pH on the partition of inorganic phosphate between water and oxidized mud or its ash. Limnol. Oceanogr., **3**: 318–326.

MORTIMER, C. H. 1941. The exchange of dissolved substances between mud and water in lakes. J. Ecol., **29**: 280–329.

———. 1942. The exchange of dissolved substances between mud and water in lakes. J. Ecol., **30**: 147–201.

ODUM, H. T. 1956. Primary production in flowing waters. Limnol. Oceanogr., **1**: 102–117.

OHLE, W. 1937a. Kolloidgele als Nährstoffregulatoren der Gewässer. Naturwissenchaften, **25**: 471–474.

———. 1937b. Kalksystematik unserer Binnengewässer und der Kalkgehalt Rügener Bäche. Geol. d. Meere u. Binnengewässer, **1**: 291–316.

———. 1955. Ionenaustauch der Gewässersedimente. Mem. Ist. Ital. Idrobiol., **8**: 221–245.

SHAPIRO, J. 1957. Chemical and biological studies on the yellow organic acids of lake water. Limnol. Oceanogr., **2**: 161–179.

STROSS, R. G. 1958. Experimentally induced changes in lakes. I. Environmental changes following lime-application to stained lakes. Ph.D. thesis. Univ. of Wisconsin Library, Madison.

TRUOG, E. AND A. H. MEYER. 1929. Improvements in the Deniges colorimetric method for phosphorus and arsenic. Ind. & Engr. Chem., Anal. Ed., **1**: 136–139.

WATERS, T. F. 1957. The effects of lime application to acid bog lakes in northern Michigan. Trans. Amer. Fish. Soc., **86**: 329–344 (1956).

——— AND R. C. BALL. 1957. Lime application to a soft water, unproductive lake in northern Michigan. Jour. Wildl. Mgmt., **21**: 385–391.

ZICKER, E. L. 1955. The release of phosphorus from bottom muds and light penetration in northern bog lake waters as influenced by various chemicals. Ph.D. thesis. Univ. of Wisconsin Library, Madison.

OXYGEN CONSUMPTION OF BIVALVE PREY AND THEIR ATTRACTIVENESS TO THE GASTROPOD, *UROSALPINX CINEREA*[1]

John W. Blake

University of North Carolina, Department of Zoology, Chapel Hill
and Institute of Fisheries Research, Morehead City, North Carolina

ABSTRACT

Relative attractiveness of *Crassostrea virginica* and *Modiolus demissus* to *Urosalpinx* was studied to determine whether oxygen consumption of prey is correlated with selection by the predator. Comparisons between individuals of one prey species and both species demonstrated a direct correlation between oxygen consumption (used as an index of respiratory metabolism) and number of *Urosalpinx* attracted. Four factors affecting metabolic rate of prey were studied: species, age, growth rate, and feeding. In each case the more attractive animals had a proportionately higher respiratory rate. *Urosalpinx* responded in equal numbers to groups of prey of different individual, but equal group, respiratory rates. Thus this work supports the hypothesis that a metabolic product mediates the predator's choice of a particular individual within a group of prey. Selection is independent of concentration of dissolved oxygen or carbon dioxide.

INTRODUCTION

It has been demonstrated frequently during the past several decades that the common Atlantic oyster drill, *Urosalpinx cinerea* (Say) (Family Muricidae), exhibits a positive chemotactic response to bivalve prey. Federighi (1931) first reported that these snails are more responsive to living than to freshly killed oysters, and that in laboratory experiments they will crawl around freshly killed oyster meats to reach living spat. Sizer (1936) learned that neither freshly excised oyster meats nor their watery suspensions would influence orientation of drills, and suggested that the attractant was a product of active metabolism present in excretions but not occurring to any appreciable extent in dead tissues. In field and laboratory studies, Haskin (1940, 1950) demonstrated that *Urosalpinx* would orient toward sea water which had passed over living oysters and thus proved that substances released by oysters and carried in the sea water attract drills. He discovered that although younger oysters were generally more attractive, older slower growing oysters from brackish water, when transplanted to areas with better growing conditions, attracted a conspicuously larger number of drills. Stauber (1943) confirmed this and added the observation that confinement of prey in aquaria for extended periods reduced their attractiveness. Janowitz (1956) found recently fed oysters more attractive than oysters from "storage," and oysters feeding on the flagellate *Cryptomonas* more attractive to drills than those fed on the diatom *Skeletonema* or *Chlorella*-like organisms.

A wide variety of animal food species other than oysters has been listed for *Urosalpinx*, and the fact that drills do not invariably choose one species or age in preference to another has been reported by a number of authors (see review by Carriker 1955). Although relative abundance and accessibility of food organisms undoubtedly play a part, other more impelling factors underlying this variation in food selection are poorly understood.

The present investigation was undertaken to determine whether a correlation could be demonstrated between the metabolic rate of prey and their relative attractiveness to *Urosalpinx*, thus testing Sizer's (1936) theory that the attracting substance is a metabolic end product.

It is an especial pleasure to acknowledge

[1] This investigation was supported in part by a U. S. Fish and Wildlife Service grant, Federal Contract No. 14-19-008-2376, and is a portion of a thesis submitted to the University of North Carolina in partial fulfilment of the requirements for the degree of Master of Arts.

the stimulating counsel of Dr. Melbourne R. Carriker throughout this study. The author is also indebted to Dr. A. F. Chestnut, Director of the Institute of Fisheries Research, and to the staff for their many courtesies.

MATERIALS AND METHODS

Specimens of *Urosalpinx cinerea* (Say) were collected on oyster reefs along the shores of Bogue Sound, North Carolina, in the Morehead City-Beaufort area, and were maintained in the laboratory in running sea water with an ample supply of food species, mainly *Crassostrea virginica* (Gmelin). The drills while in the laboratory added new shell and oviposited freely. Their young hatched and grew at a rate comparable to that of those in the field. The drills used in the experiments were 18–25 mm in shell height and were picked at random from the laboratory population; these medium size individuals were most common in the collections.

Two species were utilized as prey. Specimens of the eastern oyster, *C. virginica,* were collected from the reefs at the northwest corner of Pivers Island, Beaufort. In this area growth was rapid throughout the summer as evidenced by the thin razor-sharp edges of the valves. Slower growing individuals, distinguished by blunt thick-edged valves, were gathered along the western edge of Pivers Island in an area characterized by little circulation and heavy siltation. Atlantic ribbed mussels, *Modiolus demissus* (Dillwyn), were also collected on the Pivers Island reefs. Pelecypods used in all experiments, unless otherwise indicated, were collected the day they were used. Those held in the laboratory were undoubtedly less well fed than in their natural habitat since organisms settling within the pipes of the sea-water system used a great deal of the food that might otherwise come through. Only prey with apparently clean shells were employed in order to minimize effects of the metabolism of minute encrusting organisms. Age of the bivalves is given by year classes as follows: those setting during the summer of 1956 when the experiments were performed were considered to be zero years old; those setting during 1955, one year old; and those in 1954, two years of age. It was impossible to distinguish among earlier year classes with any degree of certainty. The slower growing "stunted" oysters were estimated to be two years of age, although they approximated the size of the one-year group from the area of rapid growth.

The experimental apparatus, patterned after that of Janowitz (1956), was designed to present predators with a choice between two water currents, one from each of two aquaria that contained those animals or materials whose attractive properties were to be compared. Sea water from the laboratory system, constantly running through a hard rubber pump and polyethylene piping, flowed into a large beaker and was siphoned out to supply the prey tanks. All connections were of glass and rubber tubing. Since water flowed in faster than it was siphoned out, the beaker continuously overflowed and provided a constant water-head; the pressure variations inherent in the laboratory system were thus eliminated. The water was divided into two equal streams, one flowing into each of the glass prey aquaria. After passing over the prey, the water flowed into a central tank through openings just above the bottom, one at the center of each end (Fig. 1). This central tank containing the predators was drained by constant-level check siphons which took water from near the bottom. It was constructed of glass and slate and was 12″ × 12″ × 18″ in dimension.

Neutral red dissolved in sea water flowing through the experimental apparatus indicated the current velocities and patterns therein, and permitted their regulation. All traces of dye were washed away before experiments were begun.

Glass T-joints were placed in the tubing entering and leaving the prey aquaria to facilitate sampling for Winkler dissolved oxygen determinations. Decrease in dissolved oxygen content of the sea water as it passed through a prey aquarium was used as a measure of the respiratory rate of the prey; this in turn served as an index of the metabolic rate. Control trials with

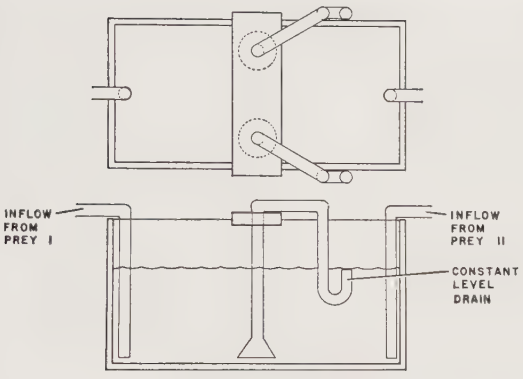

FIG. 1. Top and side views of predator aquarium (12 × 12 × 18 inches).

empty prey aquaria showed no measurable change in dissolved oxygen concentration.

In a typical experiment, prey to be compared were placed in their respective aquaria. Predator and prey aquaria were filled to correct levels, the siphons were started and flow rates were carefully equalized at about 35 ml/min. Twenty drills were placed in the predator aquarium in a double row along a line midway between the inflows from the prey tanks. Alternate snails were placed with their anterior ends toward opposite inflows. The room was darkened except for two 100-w incandescent lamps, one centered over each half of the predator aquarium. Equal light-intensity gradients around inflows from prey tanks were thus assured. The lamps caused no measurable heating of the water during the course of an experiment. Each experiment consisted of four 30-min trials. At the end of each trial, positions of the predators were noted and water samples withdrawn for oxygen analyses. Before the next trial the apparatus was flushed with fresh sea water and the organisms in the two prey aquaria were interchanged.

RESULTS

The salinities between 34.5–36.5‰ and temperatures between 25.0–30.0°C encountered during the course of the experiments, were well within the physiological tolerances of the drills.

Responses of the drills to the attractant were characteristic. A few moments after being placed in the center of the predator aquarium the responsive individuals began moving. At first they circled or wandered randomly, possibly confused by the mixing of the two opposing currents in this area. After several minutes a choice was made, and they then followed a nearly straight path to one or to the other of the inflows from the prey aquaria. As they moved toward the source of the attractant, the drills slowly rotated their shells from side to side in a horizontal plane through an arc of about 45° on either side of their path. This was apparently a searching movement of the siphon. Due to the restriction of the siphonal canal, a turning movement of the shell would be necessary to obtain alternate samples of attractant intensity to the left and right. This motion was less pronounced in some individuals than in others, and seemed to become more noticeable when snails moved out of the direct flow of the attractant. Upon arrival directly under an inflow which opened 2 cm above the bottom, the drills usually spent some time in futile attempts to follow the scent. Eventually they either remained motionless in the immediate vicinity or wandered about at that end of the aquarium.

Of those animals, usually about 50%, which did not make a definite response to water from the prey, most remained motionless. A few moved randomly near the center of the tank in a manner suggestive of the early circling movement of those drills which did respond.

Preliminary tests were negative for the factors such as phototaxis and rheotaxis, which might influence predator orientation (Table 1, Exp. 1). A comparison of young oysters with a blank proved that the apparatus demonstrated the response of the drills to prey (Table 1, Exp. 2). To obtain uniformly "hungry" predators, drills were subjected to a period of starvation for four months in containers where the only available food was the microscopic detritus and algae brought in through the laboratory seawater line. There was, however, no apparent difference between the responses of starved and normal *Urosalpinx*. Nor was there any appreciable difference in the re-

TABLE 1. *Response of* Urosalpinx *to respiratory rate of prey. Average values of 4 trials per experiment, 20 drills, and 10 rapidly growing individuals of each prey type per trial.*

Exp.	Prey Type	Age Years	*Ratio: Prey Type I/Type II		Predators Responding %
			O_2 Consumed	Predators Responding	
1	I Blank II Blank	– –	–	1.08	36
2	I *Crassostrea* II Blank	0 –	–	14.33	58
3	I *Crassostrea* II *Crassostrea*	0 1	1.98	2.23	55
4	I *Crassostrea* II *Crassostrea*	1 2	1.54	1.76	55
5	I *Crassostrea* II *Crassostrea*	0 2	3.33	3.58	51
6	I *Modiolus* II *Modiolus*	0 2	3.01	3.00	44
7	I *Crassostrea* II *Modiolus*	0 0	2.03	1.93	61
8	I *Crassostrea* II *Modiolus*	1 1	1.53	1.40	54
9	I *Crassostrea* II *Modiolus*	2 1	1.05	0.87	54
10	I *Crassostrea* 10 individuals II *Crassostrea* 17 individuals	0 1	1.03	0.99	61
11	I *Crassostrea* II *Crassostrea* slow growing	2 2	1.61	1.48	40
12	I Air II Blank	– –	–	0.97	39
13	I Carbon dioxide II Blank	– –	–	1.15	43
14	I *Crassostrea* II *Crassostrea* starved	1 1	1.97	3.92	45
15	I *Crassostrea* starved and fed II *Crassostrea* starved	1 1	1.53	0.94	53

* A ratio equal to 1.00 indicates equal values for Prey Types I & II.

sponse of young (4–5 mm shell height) and old (31–32 mm) drills. Consequently, medium sized (18–25 mm) drills were used.

Decrease in respiratory rate within one species of prey with age was accompanied by a corresponding decrease in response by the drills (Exp. 3–6, 10, see also Table 2), differences in respiration between prey species (Exp. 7, 8) were also found to be closely related to response by the drills; *Crassostrea* with its higher rate was preferred to *Modiolus*. Variations in growth

TABLE 2. *Decrease in oxygen concentration in sea water flowing over prey*

Prey Species	Age Years	Condition	Range of O_2 Consumed ml/L
Crassostrea virginica	0	rapid growth	1.73–1.97
	1	rapid growth	0.93–1.22
	2	rapid growth	0.53–0.71
	2	slow growth	0.38–0.45
	1	starved	0.48–0.63
	1	starved then fed	0.69–0.85
Modiolus demissus	0	rapid growth	0.85–1.01
	1	rapid growth	0.58–0.66
	2	rapid growth	0.26–0.35

and respiratory rates within one species of prey were paralleled by the response of the predators (Exp. 11). Drills presented with a choice between groups of prey of different species (Exp. 9) or ages (Exp. 10) with the same total respiratory rates, responded in equal numbers to each, thus indicating that the drills' criterion of choice is associated with respiratory rate of prey rather than with species or age *per se*. This association is emphasized by the correlation coefficients for experiments which compare prey of the same species (Exp. 3–6, 10 and 11) and of separate species (Exp. 7–9). In the first instance $r = 0.91$, $n = 28$; in the second, $r = 0.92$, $n = 12$. Both are significant at the 1% level. The "t" test of independence, applied to the two series of experiments shows that these two species of prey can be considered identical as far as the correlation between it in respiratory rate and relative attractiveness is concerned; that is, the drills when choosing between these prey, do so in direct proportion to respiratory rate regardless of species.

One-year-old oysters starved for 25 days in running sea water in the laboratory were compared with oysters of the same age collected in the same locality on the day of the experiment. The freshly collected oysters were more attractive as prey and were consuming oxygen at a higher rate than the starved group (Exp. 14). A suspension of the green alga, *Nannochloris atomus*, was fed to one group of starved oysters over a period of 4 hr. When these oysters were compared with the group of continuously starved individuals, it was found that, although their oxygen consumption had increased slightly, they were no more attractive to drills (Exp. 15).

Two additional experiments were carried out to determine whether the two simplest respiratory variants, oxygen and carbon dioxide, attracted drills. Air bubbled through one prey tank increased the dissolved oxygen concentration but failed to affect drill orientation (Exp. 12). Carbon dioxide evolved from dry ice similarly had no effect (Exp. 13).

Oxygen consumption for typical one- and two-year-old individual oysters was also calculated per unit dry weight of tissue. Freshly shucked meats were dried to a constant weight at 70°C. The oxygen decrease was computed for the volume of water which had passed over the oysters during a 30-min trial. Fast growing, one- and two-year-old oysters were found to consume 51.7 and 13.0 ml O_2/hr/10g respectively.

The consistent correlation of predator response and respiratory rate throughout the entire series of experiments is shown in Figure 2. The calculated correlation coefficient of 0.75 shows a linear relation, significant at the 1% level.

DISCUSSION AND CONCLUSIONS

In the experiments described here, an average of only 50% of the drills exposed to the stimuli reacted. Haskin (1950) found that on the average 42% responded, while Janowitz (1956) and Carriker (1957) both found that 75% of the drills was the highest proportion that would react in any one experiment. Inactivity may be due to two causes: the stimulus may be inadequate to evoke a response from the less perceptive individuals, or some individuals may be in a physiologically unresponsive condition. Consequently, the results were analyzed on the basis of the number of individuals making each of the alternative choices. In general the number of unresponsive drills increased as the strength of stimulus diminished (cf. Table 1, Exp. 1 and 2).

No attempt was made to standardize the time at which experiments were performed,

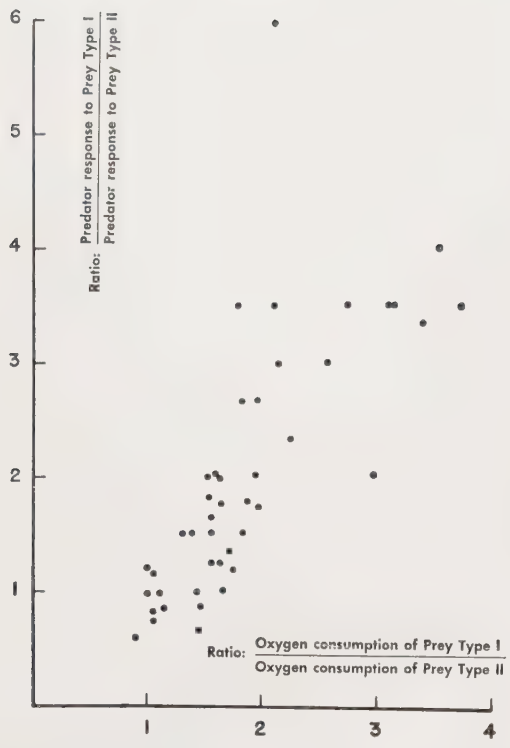

FIG. 2. Scatter diagram, prey oxygen consumption *versus* predator response. Data from Experiments 3–11, 14, 15; four trial per experiment.

except that all trials were carried out after dark in order to equalize lighting conditions. Although Brown (1954) reported that oysters showed distinct rhythmic activity associated with tidal cycles, the "natural" periodicities he observed were of small amplitude and unlikely to be significant in the present experiments. Loosanoff and Nomejko (1946) found that oysters are active and feed over 90% of the time in their natural habitat regardless of diurnal and tidal cycles.

Inasmuch as the snails receive neither reward nor punishment during the course of the experiments, there was little chance of their learning a "correct" response. Had some unrecognized reinforcement been present, it is unlikely that the drills would have had sufficient opportunity to associate it with a particular response. The minimum number of trials required for a snail to learn the simplest association appears to be about 60 (Thompson 1916, with *Physa gyrina;* Garth and Mitchell 1926, with *Rumina decollata*); other workers have found many more trials necessary. There is some evidence that another muricid drill, *Ocinebra japonica,* will usually continue to attack the species of food organism that it has attacked previously (Chew and Eisler 1958). Inasmuch as individuals of *U. cinerea* used in the present experiments were fed principally on oysters, it might be suspected that they would continue to orient to oysters in preference to mussels. However, in contrast to the conclusions of Chew and Eisler, Wood (personal communication) found that *U. cinerea* which had chosen *Crassostrea virginica* in preference to *Mytilus edulis,* would choose the oysters in only 50% of the cases, when offered the same choice a second time. Neither Chew and Eisler nor Wood measured respiratory rate of prey in their experiments.

Oxygen consumption of normal *C. virginica*, probably gathered in Massachusetts ranged from 1.75 to 9.03 ml/hr/10g dry wt (Mitchell 1914). Other oysters, probably *C. virginica,* although not so stated also gathered in Massachusetts, varied from 6.45 to 15.04 ml/hr/10g dry wt (Galtsoff and Whipple 1931). In both cases the oysters were larger and were gathered in northern waters where the growing season is shorter. Their figures, therefore, presumably refer to oysters older than the North Carolina oysters used in the present experiments which were found to consume 51.7 and 13.0 ml O_2/hr/10g dry wt for one- and two-year-old oysters respectively.

The experiments confirm and make possible new interpretation of the results of a number of previous investigators. The variation of the response of drills with the age and respiratory rate of their prey (Exp. 3–6) agrees closely with the work of Haskin (1950), and the predilection of the drills for the younger prey emphasizes previous observations by many workers (Carriker 1955). As is also suggested by Haskin's (1950) transplantation studies the comparison between fast- and slow-growing oysters demonstrates that the preference of drills is

proportional to growth rate of their prey, and in turn to the rate of respiratory metabolism. It has been shown that oyster mantle utilizing a substrate of oxaloacetate rapidly lays down new shell (Wilbur and Jodrey 1955); the observation that drills are attracted to oxaloacetic acid 10^{-9} to 10^{-7} molar (Janowitz 1956) suggests that drills may be able to detect the presence of this substance near a rapidly growing, or metabolizing, oyster. Janowitz' tests of other tricarboxylic acid-cycle intermediates should be confirmed and extended to other metabolically important materials.

Analysis of the relation between the characteristic variations in respiratory metabolism and relative attractiveness within (Exp. 3–6, 10 and 11) and between (Exp. 7–9) prey species, indicates that this correlation is significant in both instances. In like manner, the fact that when presented with a choice between groups of prey of different species (Exp. 9) or ages (Exp. 10) with the same total respiratory rates, the drills responded in equal numbers to each, demonstrates that oxygen consumption is a valid index of attractiveness for the ages and species here tested. It must be emphasized that respiratory rate of prey is not considered to be the motivating factor, but rather an easily measured index of the attractiveness of the organism to the predator; in other words, relative attractiveness and respiratory rate are similarly correlated with the actual predator stimulus. This relation may vary from one species to another in that the ratio between respiratory rate and concentration of attractant may vary, or different species may emit different kinds of attractants. Thus, the reported preferences of *Urosalpinx* for prey species other than oysters (Carriker 1955) may be indicative of either quantitative or qualitative differences in attractants.

Several authors have investigated the alternatives of qualitative or quantitative stimuli especially in relation to the feeding preferences of gastropods (Stehouwer 1952, Braams and Geelen 1953, van Dongen 1956), but have reached no definite conclusion as to which is applicable. Apparently this question can only be resolved through isolation and identification of the active principles involved. Recent identification of the so-called "secondary" plant substances and their attractant or repellent effects on phytophagous insects (Fraenkel 1959) has indicated that plants within a given family may be characterized by the same substance and be fed upon by the same insect species (*i.e.* a quantitative difference). Isolation and identification of the substance(s) mediating the feeding responses of *Urosalpinx* to various prey would provide similarly definitive data.

The highly significant correlation between oxygen consumption, as an index of respiratory metabolism, and drill response in Experiments 3–11 lends support to Sizer's (1936) hypothesis that the attracting principle is a metabolic product present in prey excretions. It would, therefore, follow that variation in feeding of prey would be reflected in their excretory products and consequently in their relative attractiveness to drills.

Experiment 14 provides a step toward confirmation of this premise. Starved oysters were, as would be expected, considerably less attractive than freshly collected oysters and consumed less oxygen. Mitchell (1914) also noted this decrease in oxygen uptake. Presumably lack of food was responsible for the lessened metabolic activity and resulted in less or different end products. This confirms and explains the observations of Stauber (1943) and of Janowitz (1956), both of whom reported that prey held in aquaria for extended periods are less attractive than recently fed individuals. It is not obvious why oysters in Experiment 15, which had been fed briefly after a long period of starvation, were no more attractive than a continuously starved group. Oxygen consumption did increase slightly, as was the case in the experiments of Galtsoff and Whipple (1931) in which they fed oysters a diluted glucose solution. It is possible that digestive processes in starved oysters are not as rapid as those in normal animals, and therefore attractive metabolic end products were not released in appreciable quantities during the experiments; or it may be that *Nannochloris*

atomus, if digested, releases a small quantity of attractive end products. It has also been suggested (Rae, personal communication) that the attractive factor may be an indigestible fraction of some food, and is not present to any appreciable extent in this alga. In a series of experiments Janowitz (1956) observed that oysters fed on *Cryptomonas* grew most rapidly and were most attractive to *Urosalpinx,* whereas oysters fed on *Skeletonema* grew somewhat more slowly and were less attractive. Wells (1958) found that most *Crassostrea virginica* attacked by the gastropod *Fasciolaria hunteria* showed chipped areas on the dorsoposterior margin of the shell, the part closest to the excurrent chamber. This seems to indicate attraction to substances, perhaps metabolic end products, in the exhalant current.

With further refinements, experiments relating growth rates and nutrition of prey to attractiveness may provide valuable clues to the identity of the attractant substance. Although the technical problems are formidable, comparative chemical analyses of the metabolic end products of prey of differing degrees of attractiveness would be most profitable.

REFERENCES

Braams, W. G., and H. F. M. Geelen. 1953. The preference of some nudibranchs for certain coelenterates. Arch. Neerl. Zool., **10:** 241–264.

Brown, F. A., Jr. 1954. Persistent activity rhythms in the oyster. Amer. J. Physiol., **178:** 510–514.

Carriker, M. R. 1955. Critical review of the biology and control of oyster drills *Urosalpinx* and *Eupleura.* Spec. Sci. Rep. U. S. Fish Wildl. Serv., Fisheries No. 148.

———. 1957. Preliminary study of behavior of newly hatched oyster drills, *Urosalpinx cinerea* (Say). J. Elisha Mitchell Sci. Soc., **73:** 328–351.

Chew, K. K., and R. Eisler. 1958. A preliminary study of the feeding habits of the Japanese oyster drill, *Ocinebra japonica.* J. Fish. Res. Bd. Canada, **15:** 529–535.

Federighi, H. 1931. Studies on the oyster drill (*Urosalpinx cinerea* Say). Bull. U. S. Bur. Fish., **47:** 83–115.

Fraenkel, G. S. 1959. The raison d'etre of secondary plant substances. Science, **129:** 1466–1470.

Galtsoff, P. S., and Dorothy V. Whipple. 1931. Oxygen consumption of normal and green oysters. Bull. U. S. Bur. Fish., **46:** 489–508.

Garth, T. R., and M. P. Mitchell. 1926. The learning curve of a land snail. J. Comp. Psychol., **6:** 103–114.

Haskin, H. H. 1940. The role of chemotropism in food selection by the oyster drill, *Urosalpinx cinerea* Say. Anat. Rec., **78:** 95.

———. 1950. The selection of food by the common oyster drill, *Urosalpinx cinerea* Say. Proc. Natl. Shellfish. Assoc., **1950:** 62–68.

Janowitz, E. R. 1956. Food selection by *Urosalpinx cinerea* (Say). Paper presented at Conv. Natl. Shellfish. Assoc. (Based on undergraduate thesis, Rutgers Univ. 1956).

Loosanoff, V. L., and C. A. Nomejko. 1946. Feeding of oysters in relation to tidal stages and to periods of light and darkness. Biol. Bull., **90:** 244–264.

Mitchell, P. H. 1941. The oxygen requirements of shellfish. Bull. U. S. Bur. Fish., **32:** 207–222.

Sizer, I. W. 1936. Observations on the oyster drill with special reference to its movement and to the permeability of its egg case membrane. Unpublished Report, U. S. Bur. Fish.

Stauber, L. A. 1943. Ecological studies on the oyster drill, *Urosalpinx cinerea,* in Delaware Bay, with notes on the associated drill, *Eupleura caudata,* and with practical consideration of control methods. Unpublished Report, N. J. Oyster Res. Lab.

Stehouwer, H. 1952. The preference of the slug *Aeolidia papillosa* (L.) for the sea anemone *Metridium senile* (L.) Arch. Neerl. Zool., **10:** 161–170.

Thompson, Elizabeth L. 1916. An analysis of the learning process in the snail *Physa gyrina* Say. Behav. Monogr., **3:** 1–89.

Van Dongen, A. 1956. The preference of *Littorina obtusata* for Fucaceae. Arch. Neerl. Zool., **11:** 373–386.

Wells, H. W. 1958. Predation of pelecypods and gastropods by *Fasciolaria hunteria* (Perry). Bull. Marine Sci. Gulf & Caribbean, **8:** 152–166.

Wilbur, K. M., and Louise H. Jodrey. 1955. Studies on shell formation V. The inhibition of shell formation by carbonic anhydrase inhibitors. Biol. Bull., **108:** 359–365.

FIRST-YEAR GROWTH OF THE WALLEYE, *STIZOSTEDION VITREUM VITREUM* (MITCHILL), AND ASSOCIATED FACTORS IN THE RED LAKES, MINNESOTA[1]

Lloyd L. Smith, Jr.

University of Minnesota, St. Paul, Minnesota

and

Richard L. Pycha

U. S. Fish and Wildlife Service, Ann Arbor, Michigan

ABSTRACT

First-year growth of the walleye, *Stizostedion vitreum vitreum* (Mitchill), was studied from 4,544 fish collected during the first summer of life in the Red Lakes in six seasons and from back calculations on 4,474 fish one year or more in age representing 17 year classes. Sexes did not differ in growth rate. Growth within the season varied greatly in different years and total growth deviated as much as 15.3% from the mean of the entire period. Water temperature, size of brood, and abundance of large walleyes and perch did not affect growth rate. Very early or late spawning influenced total growth during the season and final size and goodness or poorness of a season's growth was determined by the middle of July. Growth rate of young-of-the-year perch and total use of perch as food did not significantly influence growth. Incidence of perch in walleye stomachs ranged from 10.9 to 98.0% and spottail shiner was a more important food item in most years than perch. Total first-year growth of the walleye was apparently determined before fish became a major item in the diet.

INTRODUCTION

The walleye pike, *Stizostedion vitreum vitreum* (Mitchill), is the most important of the eight species taken in the commercial fishery of the Red Lakes, Minnesota. The wide fluctuations in its abundance during the past 25 years have been closely associated with changes in strength of individual year classes. These fluctuations have been associated with changes in the abundance of perch (*Perca flavescens*), and it was suggested by Smith and Krefting (1954) that there may be a dependence of walleyes on the perch for survival of large year classes. Since high commercial production is dependent on strong year classes, the early prediction of brood strength is desirable. If the brood strength could be estimated or the factors producing strong and weak year classes evaluated during the first year of life and 5 to 6 years prior to the entrance of any brood into the catch, many advantages for economic production and possibly for population management would follow. With these facts and possibilities as a background, study of first-year walleyes was started in 1951 and carried through 1957. In addition to the data collected during these years, detailed statistics of abundance, yield, and fishing effort were available from 1930 through 1957 in fishery records. Comprehensive information on spawning seasons in both upper and lower lakes from 1940 to 1957 was secured from hatchery logs. The present study deals primarily with problems of growth and the manner in which it may be affected by various factors in the lake during the first year of life. The bearing of these facts and others on survival and establishment of brood strength will be discussed in a subsequent paper.

Various workers have made substantial contributions to knowledge of first-year life in the walleye. An excellent review of literature up to 1950 is made by Eschmeyer (1950). Maloney and Johnson (1957) discussed various aspects of food and growth in two large Minnesota lakes, and other

[1] Paper No. 4199, Scientific Journal Series, Minnesota Agricultural Experiment Station, St. Paul 1, Minnesota.

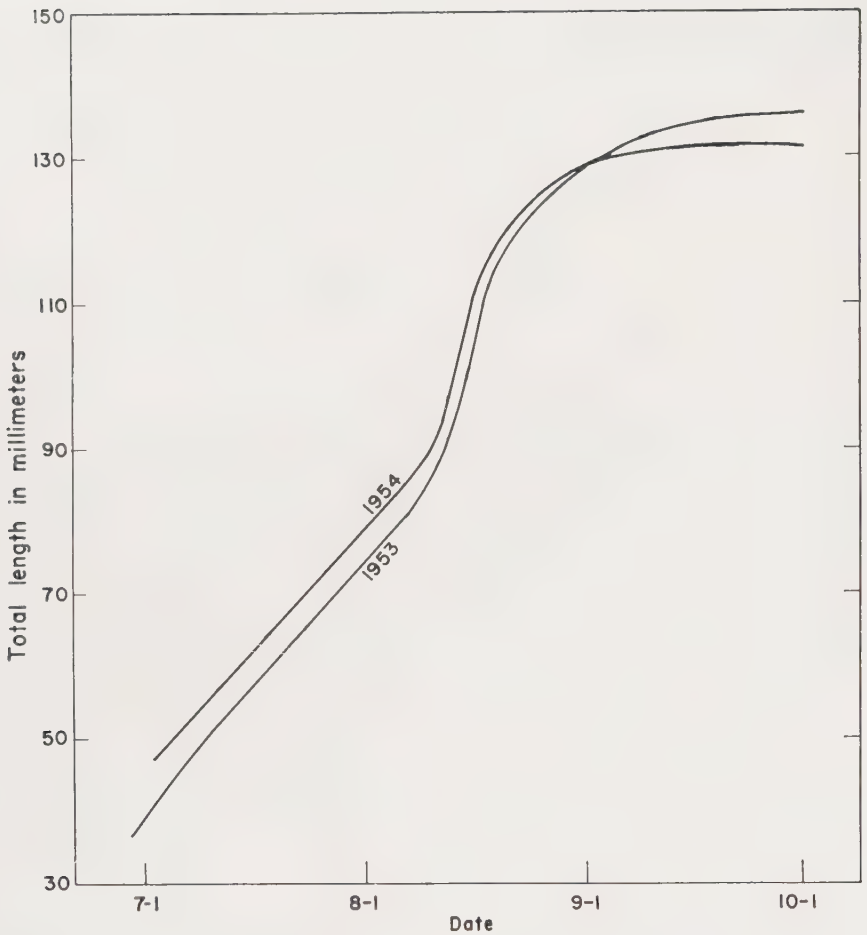

FIG. 1. Growth of young-of-the-year walleye in 1953 and 1954 showing growth compensation in mid-season.

workers have contributed recently to information on first-year growth.

The authors wish to acknowledge the assistance of M. A. Grosslein, F. E. Lux, R. J. Knapp, and J. R. Magnuson for field and laboratory assistance. Financial aid was received from the National Science Foundation and the Great Lakes Laboratory of the U. S. Fish and Wildlife Service.

RATE OF GROWTH

Determination of first-year growth characteristics of the walleye was made from 4,544 fish collected with bag seine and trawl at intervals throughout the summer season from 1951 through 1957. Average total fish lengths at the end of the first growing season in the broods of 1940 through 1956 were calculated from 4,474 fish one year or more in age caught with experimental gill nets and shore seines. These calculations were made with a nomograph constructed from a body-scale length regression based on 1,521 fish ranging from 55.9 to 561.3 mm in total length. All young-of-the-year fish and the majority of I-annulus fish used were preserved in 10% formalin before measurements were made. Some I-annulus and all older fish were measured in fresh condition. Weights of young-of-the-year fish were taken from preserved specimens.

Growth of sexes

In later years of life female walleyes are larger than males of the same age and Esch-

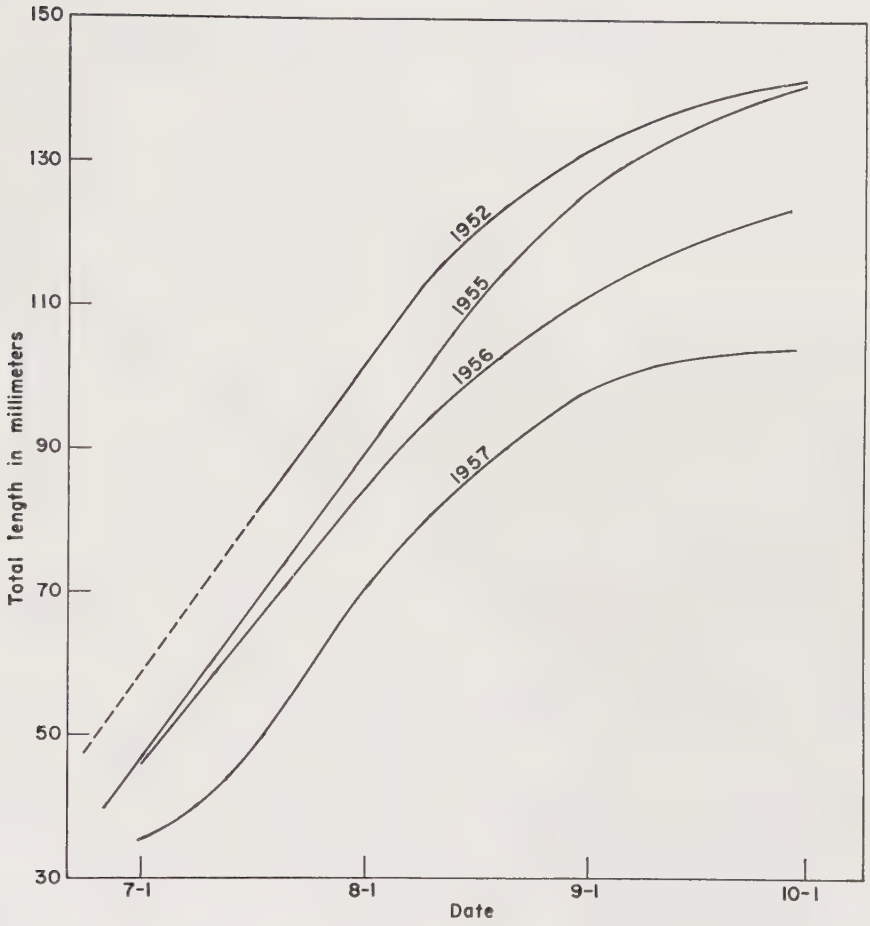

FIG. 2. Growth of young-of-the-year walleye in 1952, 1955, 1956, and 1957.

meyer (1950) shows some indication that this difference also exists during the first year. In 20 samples taken from Red Lakes on different dates throughout the 1955 summer season, there was no significant difference between the mean lengths of males and females from the individual collections. In 10 cases the average length of females was larger, in 6 it was smaller, and in 4 there was less than 1 mm difference. When lengths at I-annulus were compared in 17 year classes from 1940 through 1956, observed differences between sexes were nonsignificant. Samples of first-year fish picked from collections during the summer season of other years similarly showed no differences between sexes. Preliminary scanning of length-frequency data for the two sexes suggested that there might be a skewness in the length distribution of either sex on particular dates that would influence the relative number of larger fish in each group. Distributions were plotted for 5 year classes at first annulus and for several of the large samples during the first growing season. In some instances there appeared to be more large fish among the females, and the distribution was less concentrated about the mean than in the males. These differences were not sufficiently consistent to justify any conclusions. Since significant differences between the sexes could not be shown, all data for the first season's growth were combined for subsequent analyses.

Growth during first season

From 1952 through 1957, 380 samples of

TABLE 1. *Length of walleyes (millimeters) on various dates during first season of growth, 1951–1957*[1] *(numbers in parentheses are percentages of total season's growth)*

Date or item	Year class							Total
	1951	1952	1953	1954	1955	1956	1957[3]	
July 1	—	—	39	—	47	47	36	
			(29)		(33)	(38)	(35)	
July 10	—	—	52	57	60	59	44	
			(39)	(44)	(42)	(48)	(43)	
July 20	80	87	63	68	74	71	55	
	(58)	(58)	(47)	(52)	(52)	(57)	(53)	
July 30	—	100	72	77	87	85	69	
		(66)	(54)	(57)	(61)	(69)	(67)	
August 10	—	115	86	89	103	95	81	
		(77)	(64)	(68)	(73)	(77)	(78)	
August 20	—	124	116	118	115	104	91	
		(83)	(87)	(91)	(81)	(84)	(86)	
August 30	—	131	127	128	125	111	97	
		(87)	(96)	(99)	(88)	(90)	(93)	
I-annulus[2]	140	142	134	130	142	124	—	
Number of young-of-the-year fish	107	280	435	580	526	1,559	1,057	4,544
Number of I-annulus fish	479	59	53	166	246	66	—	1,001
Number of samples	3	8	23	41	73	117	118	383

[1] Taken from smoothed curves of sample means.
[2] Year classes 1952–1956 calculated from I-annulus fish; 1951, from 479 fish of all ages.
[3] Percentages estimated from curve with last point on September 20.

first-year walleyes were taken at intervals during the growing season. In 1951 three samples were taken on July 22–23. The mean lengths of the fish in these collections were plotted and smoothed curves drawn to describe the growth of the different year classes (Figs. 1, 2, Table 1). There was considerable variation in both the rate and pattern of growth in the different seasons. The greatest size differences in mean total length were attained by August 10. On that date in different years there was a maximum spread of 34 mm between 81 mm in 1957 and 115 mm in 1952. By the end of the growing season the range of mean length had decreased to 18 mm between 124 mm in 1956 and 142 mm in 1952. In the 17-year period 1940 to 1956, the average length at the end of the first year of growth was 137 mm with a range of 32 mm between 118 and 150 mm (Table 2).

The rate of growth in most seasons was relatively constant from the latter part of June through the first week in August. After this period in years of moderate or good early growth there was a gradual decline in rate (1951, 1952, 1955, 1956). During years of poor early growth (1953, 1954, 1957) growth rate increased during mid-season and then declined. This acceleration started during the third week of July in 1957 but not until the first week of August in 1953 and 1954. In the latter two years this increased rate extended through August and then tapered off rapidly. It overcame to a substantial degree the effects of slow early growth or late start from delayed hatching. This growth compensation brought final sizes for the season close to the average for the period. In 1957 growth through July 15 was at the slowest rate of the 7 years observed and the moderate compensation left the total length on September 20 far below average.

Since the maximum range in mean length on August 10 in a 7-year period was approximately twice that at the end of the growing season in 15 years and 40% greater than that in two years of a 17-year period, there may be even larger variations between years in mid-season than those noted. The type of growth compensation observed par-

TABLE 2. *Length of walleye in Red Lakes at first annulus and percentage deviation from mean annual increment of I-annulus fish and all other ages in various calendar years, 1940–1956 (1957 estimated)*

Year class	Number of I-annulus fish	Length in mm	Percentage deviation	
			I-annulus fish	Older fish[2]
1940	41	140	+2.0	—
1941	40	135	−1.6	+6.6
1942	12	118	−15.0	−24.8
1943	41	136	−0.9	+22.3
1944	238	142	+3.5	+19.4
1945	148	137	0.0	−19.7
1946	736	141	+2.8	+8.7
1947	748	143	+4.2	+30.7
1948	422	150	+9.0	+25.0
1949	593	146	+6.3	+2.4
1950	386	136	−0.8	−42.2
1951	479	140	+2.0	+3.4
1952[1]	59	142	+3.4	−1.8
1953	53	134	−2.4	−31.9
1954	166	130	−5.4	−4.5
1955	246	142	+3.4	+7.5
1956	66	124	−10.0	−1.4
1957	—	(106)	(−22.0)	—
Mean		137		
Total	4,474			

[1] Calculation of first-year growth 1952–1956 made from I-annulus fish taken in seines; other years from older fish.

[2] Calculation made after Hile (1941) from 5,152 II-annulus and older fish caught in experimental nets and spawning runs 1949–1957.

ticularly in 1953 and 1954 probably occurs in most years when fish are substantially smaller than average size in June and July. First-year growth of fish from Oneida Lake plotted from the data of Raney and Lachner (1942) shows some indication of this compensatory growth.

The percentage of total first-year growth in length which has been completed on any particular date varies considerably in different seasons. By July 20 in all years observed 47 to 58% and in most years more than 50% of the season's increment is completed. At the end of the first 10 days in August, 64 to 78% and, by August 30, 87 to 99% of growth has been made. In years of greatest first-year growth 12 to 13% of the total was added after September 1.

The mean length of Red Lakes walleyes at the end of the first growing season in 17 year classes was 137 mm. This length was somewhat shorter than that reported for Lake of the Woods (Carlander 1945) where fish at I-annulus were 162 mm total length. Hile (1954) found total length at I-annulus in Saginaw Bay to be 162, 170, and 175 mm in different collections. Deason (1933) reports a length of 91 mm standard length and Van Oosten (1948) reported 152 mm total length in Lake Erie. Other authors have found varying growth rates within these ranges from comparable waters.

Annual variations in growth

As has been pointed out above in connection with young-of-the-year, and by numerous authors working with larger fish, there is a marked variation in annual growth increment of walleyes. The growth of walleyes from Red Lakes during the first year of life deviated from the mean growth of all years of the 17-year period, 1940–1956, by as much as 15.3% and the maximum variation between different seasons was 24.0% of the mean annual increment[2] (Table 2). The greatest growth was made in 1948 and the smallest in 1942. When the percentage deviation from the mean annual increment of the I-annulus fish was compared with that of all fish in older age groups in corresponding calendar years from 1941 through 1956, a low correlation ($r = .47$, $p = .073$) was obtained. This coefficient was considerably lower and less significant than that noted by Hile (1954). In some years trends were in opposite directions and in others there was no consistency in degree of change between the groups. It must be concluded that the factors which control the growth of I-annulus fish and older fish are different or do not have equal effect on both groups.

Factors associated with growth

Growth of walleyes during the first year of life is determined by a combination of factors which may include temperature, food availability, time of spawning, abundance of older fish, and abundance of other small competing or forage species.

Temperature—Since temperature is close-

[2] When estimated total increment of the 1957 season is included, maximum range is approximately 31%.

TABLE 3. *Total length in millimeters of walleyes and perch at the end of the first growing season*

Year class	Length of walleye	Length of perch
1940	140	62
1941	135	65
1942	118	65
1943	136	66
1944	142	64
1945	137	63
1946	141	68
1947	143	61
1948	150	63
1949	146	63
1950	136	54
1951	140	63
1952	142	56
1953	134	75
1954	135	68
1955	142	75
1956	124	68
1957	106[1]	—

[1] Estimated.

ly associated with growth in poikolothermic organisms, its influence on the growth of young walleyes was examined by comparing the summation of air-degree days above 45°F and above 50°F with the total length at the end of the growing season as determined from back calculations on I-annulus and older fish. Comparisons were made with summations for May, June, and July; May, June, July, and August; July and August; August only; August and September; September only; the last two weeks in July, August, and September; for the 17-year period 1940–1956. Use of degree-days calculated from records of the Red Lake weather station was considered valid because the shallow, unstratified lake follows air temperature closely (Smith, *et al.* 1952). While all summations of degree-days tested showed a positive correlation with growth of young walleyes, none were significant below the 15% level. Inclusion of July data made coefficients even lower.

Size of perch—Food studies described below together with similar work by numerous authors indicate that yellow perch is a major food of walleyes and in some cases is the most important item of the diet. Comparison of the growth of young perch in Red Lakes (Pycha and Smith 1955) with the growth of young walleyes in 1952, 1953, 1954, 1955, 1956, and 1957, suggests that slow-growing perch were associated with fast-growing walleyes. The 1952 year class of walleyes ranked second in growth rate during a 17-year period, while the same year class of perch had the slowest growth during the same period. In contrast the 1953 and 1954 broods of walleyes were among the slowest growing broods of the period, while the perch in the same years had a growth substantially above average (Table 3). Correlation between total length of walleyes and the length of perch at the end of the first growing season from 1943 to 1956, however, was non-significant ($r = -.21$, $p = .50$). While this comparison does not lend support to the observations based on recent year classes, the habits and food analyses suggest that the smaller perch would be more readily available to the walleyes over a longer period and therefore might contribute to a faster growth rate. Actual utilization of perch by walleyes was greatest in years when perch were smallest and least when they were large, as shown below in the discussion of food habits.

Abundance of population—Various observations of walleye growth in rearing ponds suggest that growth of young fish in any season is inversely related to the density of the population (Dobie 1956). In Red Lakes an estimate of strength of 15 year classes was made from the examination of approximately 25,000 fish taken from the commercial fishery, experimental gill nets, and shore seines over a 9-year period. Corrections were made for fishing effort and general levels of abundance.[3] The relative strength of the year classes ranged between 5% and 90.9% of the strength of the 1947 brood which was used as a base. Comparison of growth of young fish in different years with the brood strength in the same season showed a superficially positive but nevertheless non-significant relationship. It

[3] Smith, Lloyd L., Jr., and Richard L. Pycha (MS). Influence of growth and strength of year class of walleye on commercial availability in the Red Lakes, Minnesota.

therefore appears that at population levels existing in Red Lakes the strength of the brood has no demonstrable influence on rate of growth during the first year. Since larger perch and walleyes utilize young perch and other small-fish items taken by fingerling walleyes, competition could be a factor in growth as well as survival. Comparison of abundance levels of commercially available perch and walleye (Smith and Krefting 1954; Smith and Pycha MS) with growth of young walleyes showed no relationship.

Date of spawning—From 1941 through 1957, dates of spawning runs and of peak egg production at Waskish hatchery on Upper Red Lake were compared to total growth during the following summer season. Spawning started as early as April 5 and as late as May 2 and continued from 10 to 28 days in various years. Since river water is used in the hatcheries, dates of hatching recorded there are assumed to be approximately the dates when naturally spawned eggs would hatch in the streams. Hatching was completed as early as May 9 and as late as May 29. The influence on growth of the date when river hatching was completed is not clear, perhaps because the growth compensation noted above tends to overcome a late start or because a substantial portion of fish are spawned in the lake where hatching dates are different. Only very early or very late hatching was associated with the size attained by fish at the end of the first growing season. In the two seasons of greatest growth, hatching was over before May 10, while in the two years of poorest growth the hatching was not completed until May 26 and May 29. In the years when detailed growth data through the season are available, the size of the fish in early July appears to be closely related to the time of spawning with the slow years having late hatching and the fast-growing years having early hatching dates. Available information, although inconclusive, suggests that dates of hatching influence size of July fish appreciably but total seasonal growth only when they are very early or very late.

LENGTH-WEIGHT RELATIONSHIP

The length-weight relationship of young walleyes was determined from 1,246 fish which covered a range of 31 to 135 mm in total length, and were collected in the four-year period 1952–1955. The relationship was adequately expressed by the equation

$$W = 0.000007058\ L^{3.03811}$$

where W = weight in grams and L = total length in millimeters.

The relative plumpness of fish has been expressed in various ways for the purpose of comparing different groups of fish (Le-Cren 1951). In the present study the general condition of fish has been expressed as a relative condition factor calculated from the formula

$$K_n = \frac{W}{W_1}$$

where W = weight at capture and W_1 = weight calculated from the length-weight equation.

Since use of relative condition factor (K_n) permits the comparison of fish with reference to plumpness without consideration of factors associated with length, it is useful in detecting differences between fish at different times of year and in different years or between fish of different sizes on the same date. The average relative condition factors for all fish taken each year in the period 1952–1957 varied somewhat (0.992, 1.060, 0.995, 0.948, 0.934, and 0.991 for respective years) but without any consistent relationship to food utilization or rate of growth.

When average value of K_n for successive 15-day periods through the growing season was calculated, there appeared to be a lowering of condition as the season advanced, especially during seasons of slower average growth (Table 4).

Direct observation suggested that the larger fish among those taken on any one date were proportionally heavier than the smaller ones. Therefore, the average conditions (K_n) of all fish larger than the mean length and of those smaller than the mean in each sample were calculated separately.

TABLE 4. *Average relative condition factor (K_n) of young walleye pike above and below average length during various periods of the growing season 1952–1957*

Year and period	Condition factor (K_n)			
	Number of fish	Average	Above mean length	Below mean length
1952				
June 16–30	—	—	—	—
July 1–15	—	—	—	—
July 16–31	49	0.999	0.995	1.007
August 1–15	50	0.935	0.997	0.974
August 16–31	—	—	—	—
1953				
June 16–30	10	1.091	1.115	1.075
July 1–15	113	1.083	1.104	1.057
July 16–31	126	1.076	1.084	1.069
August 1–15	102	1.031	1.049	1.014
August 16–31	53	1.043	1.049	1.035
1954				
June 16–30	—	—	—	—
July 1–15	—	—	—	—
July 16–31	227	1.028	1.056	0.993
August 1–15	67	1.937	0.966	0.908
August 16–31	53	0.982	1.001	0.889
1955				
June 16–30	47	0.929	0.940	0.921
July 1–15	127	0.943	0.950	0.938
July 16–31	158	0.950	0.981	0.923
August 1–15	27	0.943	0.981	0.902
August 16–31	22	0.938	0.892	1.004
1956				
June 16–30	9	1.046	1.015	1.030
July 1–15	615	0.958	0.911	0.941
July 16–31	476	0.914	0.943	0.885
August 1–15	278	0.869	0.914	0.829
August 16–31	163	0.866	0.877	0.854
1957				
June 16–30	—	—	—	—
July 1–15	506	1.045	1.036	1.024
July 16–31	266	0.975	0.972	0.979
August 1–15	150	0.909	0.891	0.932
August 16–31	67	0.931	0.965	0.895

These factors were weighted and combined with those of other samples by 15-day intervals (Table 4). In 24 comparisons between the condition of larger fish and smaller fish, the larger group was heavier in 19 cases. Application of sign test indicates a probability level of 1%. When single large samples were considered separately, the contrast between large and small groups was more marked. The average condition factor (K_n) of fish in separate quartiles of the distribution of individual samples showed a progressive increase from small to larger fish in most cases. In all samples the first quartile was relatively lighter than the fourth.

GROWTH AND FOOD UTILIZATION

During the course of this study the stomachs of 3,652 young-of-the-year walleyes were examined for food selection. The samples were taken in six seasons at four stations on the lake. Of the total number 17.4% were empty and the remainder had a variety of food items among which fish of various species and copepods predominated. The most important food organisms in fish up to 50 mm in length were copepods and to a lesser degree cladocerans.[4] Walleyes larger than 50 mm relied heavily on forage fish and perch but all sizes used copepods and *Leptodora* in some quantities throughout the season.

Since one of the objectives of the study was to ascertain the role of perch in the growth of walleyes, utilization of perch was assessed on the basis of percentage incidence in stomachs of walleyes taking food. Where fish remains could not be identified, they were assigned to various species on the basis of the ratio of the numbers in individual species to the total of identified fish. During the six-year period 1952–1957, perch were present in 10.9 to 98% of the walleyes in different seasons (Table 5). Incidence exceeded 58.5% only in 1952 when sampling was limited. The combined percentage occurrence of other fish species, which included *Notropis hudsonius, N. atherinoides, Catostomus commersonii, Percopsis omiscomaycus, Etheostoma nigrum, Aplodinotus grunniens, Esox lucius,* and *Stizostedion vitreum,* varied from 3.5 to 42.0% in various years. The single most important forage fish item other than perch was *Notropis hudsonius.* In some years (1955, 1956, 1957) the total number of the latter species which was used exceeded the number of perch, in one year (1957) by a margin of 3 to 1. The spottail shiner (*N. hudsonius*) was used most heavily by walleyes smaller

[4] Smith, Lloyd L., Jr., and John Magnuson (MS). Food of walleyes in Red Lakes, Minnesota.

TABLE 5. *Utilization of perch and other forage fish by young-of-the-year walleye in the Red Lakes expressed as percentage of total fish taking food 1952–1957*

Year	Total fish	Percentage empty	Percentage with perch	Percentage with other fish	Percentage deviation from 18-year growth	Condition factor (K_n)
1952	100	11.0	98.0	3.5	+3.4	0.992
1953	406	23.0	32.5	37.1	−2.4	1.060
1954	400	20.0	58.5	22.1	−5.4	0.995
1955	406	15.2	22.1	28.0	+3.4	0.948
1956	1,423	19.4	46.6	37.3	−10.0	0.934
1957	917	12.5	10.9	42.0	(−22.0)[1]	0.991
Total	3,652					
Average		17.4				

[1] Estimated

than 71 mm in length and during the earlier part of the season.

Perch were important in the diet after walleyes attained a length of 51 mm and the percentage occurrence rose as they increased in size. Even in the larger fish, however, incidence rarely exceeded 50%. When rate of walleye growth was compared to utilization of perch, it was apparent that the two were not strongly related. The two years of greatest growth, 1952 and 1955, had the greatest and next to the smallest incidence of perch (98.0 and 22.1%). The year 1954, which had next to the highest utilization of perch, had next to the lowest growth rate of the 6-year period. In 1957, the year with poorest growth to September 1, incidence of perch was smallest. With the exception of 1952, when sampling was limited to two stations and four samples, the years of heaviest use of all fish as food were the poorest growth years. In 1955, when the incidence of fish in stomachs was lowest of the five years observed, growth rate of walleye was greater than in all except four years of the 18-year period for which growth data are available.

As was pointed out above, total growth for the season appears to be contingent on the size attained by the end of the first week of July, although growth compensation may overcome initial poor starts to some extent. It would appear from the available information that the extent of utilization of fish does not determine the rate of growth or the total growth of the first season.

Food selection and abundance prior to the first 10 days in July would be expected to show more correlation with total first-year growth than later food habits. During that period food of the walleye is made up largely of copepoda, cladocera, and insect larvae. In most years of slower growth there was a higher percentage of empty stomachs, but the average condition factor of all young walleyes was not consistently related to rate of growth (Table 5).

REFERENCES

CARLANDER, KENNETH D. 1945. Age, growth, sexual maturity, and population fluctuations of the yellow pike-perch *Stizostedion vitreum vitreum* (Mitchill), with reference to the commercial fisheries, Lake of the Woods, Minnesota. Trans. Am. Fish. Soc., **73:** 90–107.

DEASON, HILARY J. 1933. Preliminary report on the growth rate, dominance, and maturity of the pike-perches (*Stizostedion*) of Lake Erie. Trans. Am. Fish. Soc., **63:** 348–360.

DOBIE, JOHN. 1956. Walleye pond management in Minnesota. Prog. Fish-Cult., **18:** 51–57.

ESCHMEYER, PAUL H. 1950. The life history of the walleye, *Stizostedion vitreum vitreum* (Mitchill), in Michigan. Bull. Inst. Fish. Res., **3:** 1–99.

HILE, RALPH. 1941. Age and growth of the rockbass, *Ambloplites rupestris,* (Rafinesque) in Nebish Lake, Wisconsin. Wis. Acad. Sci. Arts, Lett., **33:** 189–337.

———. 1954. Fluctuations in growth and year-class strength of the walleye in Saginaw Bay. U.S. Fish and Wildl. Serv., Fish. Bull., **91** (56): 7–59.

LECREN, E. D. 1951. The length-weight relationship and seasonal cycle in gonad weight and condition in the perch (*Perca fluviatilis*). Jour. Animal Ecol., **20:** 201–219.

Maloney, J. E., and F. H. Johnson. 1957. Life histories and interrelationships of walleye and yellow perch, especially during their first summer, in two Minnesota lakes. Trans. Am. Fish. Soc., **85**: 191–202.

Pycha, Richard L., and Lloyd L. Smith, Jr. 1955. Early life history of the yellow perch, *Perca flavescens* (Mitchill), in the Red Lakes, Minnesota. Trans. Am. Fish. Soc., **84**: 249–260.

Raney, E. C., and Ernest A. Lachner. 1942. Studies of the summer food, growth, and management of young yellow pike-perch, *Stizostedion v. vitreum*, in Oneida Lake, New York. Jour. Wildl. Mgmt., **6**: 1–16.

Smith, Lloyd L., Jr., and Laurits W. Krefting. 1954. Fluctuations in production and abundance of commercial species in the Red Lakes, Minnesota, with special reference to changes in the walleye population. Trans. Am. Fish. Soc., **83**: 131–160.

———., Laurits W. Krefting, and Robert L. Butler. 1952. Movements of marked walleyes, *Stizostedion vitreum vitreum* (Mitchill), in the fishery of the Red Lakes, Minnesota. Trans. Am. Fish. Soc., **81**: 179–196.

Van Oosten, John. 1948. Turbidity as a factor in the decline of Great Lakes fishes with special reference to Lake Erie. Trans. Am. Fish. Soc., **75**: 281–322.

OBSERVATIONS ON THE ECOLOGY OF THE SOFT CLAM, *MYA ARENARIA*, IN A SALT POND[1]

George C. Matthiessen

Division of Marine Fisheries, Commonwealth of Massachusetts, Boston, Massachusetts

ABSTRACT

Observations were made on the ecology of the soft clam, *Mya arenaria*, in a salt water pond on Martha's Vineyard, Mass., during the summer and fall of 1958. Primary consideration was given to the tolerance of this species to low and fluctuating salinities, the effect of salinity upon rate of growth, and possible correlation between growth rate and the nature and abundance of phytoplankton.

Field observations supplemented by laboratory experimentation indicated that *Mya* longer than 2 mm can tolerate salinities less than 1‰ for at least 24 hrs and can withstand sudden salinity fluctuations of an order of 18‰. Tolerance of clams 2–25 mm in length to low salinities was found to be proportional to their size.

Growth rate of clams living under different salinity conditions ranging in mean value from 3.5‰ to 14‰ was found to vary directly with salinity. The fact that the pumping rate of clams was observed to vary directly with salinity within the limits 0–31‰ is considered significant in this respect.

Comparisons of growth rates with phytoplankton concentrations suggested that flagellates form an important source of nutrition for *Mya* and that diatoms may be of relatively little importance.

INTRODUCTION

The continued decline in abundance of clam and oyster populations in many coastal areas during the past decade has accentuated the need for more efficient methods of utilizing a potentially valuable marine resource. It has been suggested by certain investigators (Turner 1951; Loosanoff 1958) that shellfish might be raised quite profitably in enclosed or semi-enclosed salt water ponds in which optimum environmental conditions could be maintained. For example, it might be possible to exclude oceanic predators completely, to accelerate shellfish growth indirectly through the addition of fertilizer, and to eliminate the waste involved in the dispersal of shellfish larvae to inaccessible areas.

In certain respects the soft clam, *Mya arenaria*, might be ideally suited for pond culture. The ability of this species to thrive in brackish water has been recognized by shellfish investigators for many years (Belding 1930), and its tolerance to low and variable salinity conditions does not appear to be shared by its most significant and primarily oceanic predators, such as the green crab (*Carcinides maenas*), the horseshoe crab (*Limulus polyphemus*), or the boring snail (*Polinices heros*). However, the minimum salinity conditions required for a thriving, self-replenishing *Mya* population have not been established.

The objective of this investigation was to study certain factors influencing the survival and growth of members of a clam population in Oyster Pond, a salt water pond located on the south shore of Martha's Vineyard, Mass. Consideration was given primarily to the minimum salinity conditions under which this species is capable of survival, reproduction, and growth, and secondarily to its probable source of nutrition.

The author is sincerely grateful to Mr. Harry J. Turner of the Woods Hole Oceanographic Institute and Dr. George L. Clarke of Harvard University and the Woods Hole Oceanographic Institute for their advice during this project, to Mr. Anthony Provenzano of the U.S. Fish and Wildlife Service for his cooperation and suggestions, and to Mr. Thomas L. Flynn of Edgartown, Mass., for his permission to undertake re-

[1] Based upon data included in a thesis in biology submitted to the Graduate School of Arts and Sciences of Harvard University in partial fulfilment of the degree of Doctor of Philosophy.

Contribution No. 1056 from the Woods Hole Oceanographic Institute.

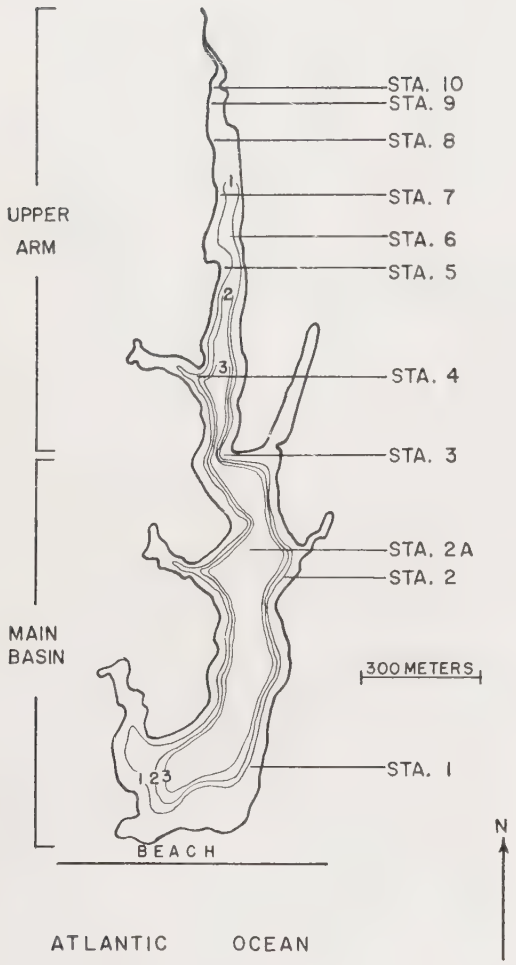

FIG. 1. Chart of Oyster Pond. Depth in meters.

search in Oyster Pond. Financial assistance in the form of a fellowship grant from the Woods Hole Oceanographic Institution is gratefully acknowledged.

DESCRIPTION OF OYSTER POND

Oyster Pond is usually separated from the Atlantic Ocean by a sand beach 50 to 100 m in width (Fig. 1). The pond is opened periodically by bulldozing a channel across the beach, resulting in a sharp increase in salinity throughout much of the pond. The pond seldom remains open for longer than two to three weeks, as wind-blown sand gradually fills the channel. Once closed, the pond water gradually freshens due to precipitation, seepage, and a stream at the extreme north end. The northern half of the pond, or upper arm (Fig. 1), is relatively shallow and bordered by wooded hills; the main basin is appreciably deeper and, due to the flat topography, is exposed to wind from all quarters.

The only species of bivalves of numerical significance inhabiting Oyster Pond are *Mya arenaria, Crassostrea virginica,* and *Macoma baltica.* The effect of the few potential predator species observed—the decapods *Callinectes* and *Panopeus* and the flatworm *Stylochus*—upon the bivalve population is uncertain but is not considered to be appreciable. A survey of the distribution of *Mya* was undertaken prior to June 16 when the pond was open and the water level low. It was observed that the population extended as far up the pond as Station 7 (Fig. 1) but no further. As no apparent change in sedimentary characteristics took place at this point, it was presumed that low salinity conditions prevented the population from advancing further.

Oyster Pond was open to the sea from June 3–June 16, 1958. After a strong southwest wind closed the inlet during the evening of June 16, it remained closed until August 27, 1958, when it was reopened by bulldozer. The strong salinity gradient that generally prevailed along the pond's axis during the period of June 16–August 27 provided a favorable opportunity to study the role of salinity in the ecology of a *Mya* population in a relatively small area, particularly as this period coincided with the population's major spawning period. After the pond closed on September 9, two northeast storms occurred in quick succession and destroyed the salinity gradient that had prevailed during the summer months. For reasons to be discussed below, homogeneity of both temperature and salinity throughout much of the pond during the autumn was accompanied neither by a homogeneous distribution of different planktonic species nor of phytoplankton abundance in general. Conditions were therefore considered ideal during the period September 18–November 6, 1958, for comparing the effects of differ-

ent phytoplanktonic forms upon the growth rate of *Mya*.

METHODS

Clams were collected from the pond population near Station 1 and placed in shallow, sediment-filled trays, 40 clams to each tray. These clams, ranging in length from 13–25 mm, were measured with vernier calipers and numbered with finger-nail polish for future identification. The trays were placed at Stations 1–10 (Fig. 1) at a depth of about one meter. The clams were measured at approximately two-week intervals during the period June 16–August 26. At least three times each week the temperature and salinity at each station was determined to the nearest 0.1°C and 0.1‰, the latter by means of hydrometer.

To compare the ability of *Mya* of different sizes to survive under presumably adverse salinity conditions, small clams 2–4 mm long were collected and placed in quart jars, approximately 100 clams to each jar. The tops of the jars were covered with plastic screening. The jars were placed next to the wooden trays at Stations 4–8 for a six-day period, after which they were inspected to record any mortalities. The experiment was then repeated.

An attempt to determine whether clams native to Oyster Pond might have developed an unusually high tolerance to low salinity conditions was made in the following manner. A sample of 60 clams, ranging in length from 18–40 mm, were collected from a tidal flat along the Vineyard Sound shoreline in an area where the salinity would not be expected to vary appreciably from 28–32‰. These clams were placed in sediment-filled trays, 20 clams to each tray, at Stations 4, 6 and 8; their growth and survival rates were compared with native Oyster Pond clams in trays at the same stations.

Supplementary studies on the tolerance of clams of different sizes to low salinities were conducted under artificial conditions. A group of 125 clams, 2–4 mm in length, were collected from an area in Oyster Pond where the bottom salinity was 18‰ and had ranged from 18–30‰ during the previous month. They were placed in a polyethylene container of distilled water, temperature 20°C, for a period of 24 hr. Every 6 hr thereafter for the next 24 hr, groups of 25 clams were removed and placed in separate containers that had a layer of sand on the bottom and were filled with water of salinity 18‰. Mortality percentage was easily determined. Upon being returned to normal (18‰) salinity, the clams either dug into the sediment within 24 hr or remained on the surface of the sediment with shells agape and failed to respond to mechanical stimulation.

This experiment was repeated with groups of clams 5–10 mm and 15–25 mm in length. In another series of experiments salinities of 1, 2 and 4‰ were used instead of distilled water with clams in the 5–10 mm size range.

Determination of the relative pumping rates of clams under different salinity conditions was made by the method described by Cole and Hepper (1954). Six 3-liter polyethylene containers with a sand substratum were filled with water of 31, 15, 7.8, 3.9, 2.0 and 1‰. Six clams, averaging 27 mm in length, were placed in each container; the containers were placed in a thermostatically-controlled water bath at 21°C (± 1°). After a 24 hr acclimatization period, a dilute solution of Neutral Red was introduced into each container, and also into a seventh container serving as control, in sufficient amount to produce an initial Neutral Red concentration of 0.0003%.

At half-hour intervals, 10 ml samples of water were drawn from each container and acidified with three drops of concentrated HCl to restore coloration. The optical density of each sample was determined by means of a Beckman spectrophotometer and converted into per cent concentration by use of a calibration curve. In this way it was possible to determine the relative pumping rate at different salinities, the presumption being that Neutral Red would be removed from the water, by adsorption to the gills, at a rate directly proportional to the pumping rate of the clams.

To study the effects of different phyto-

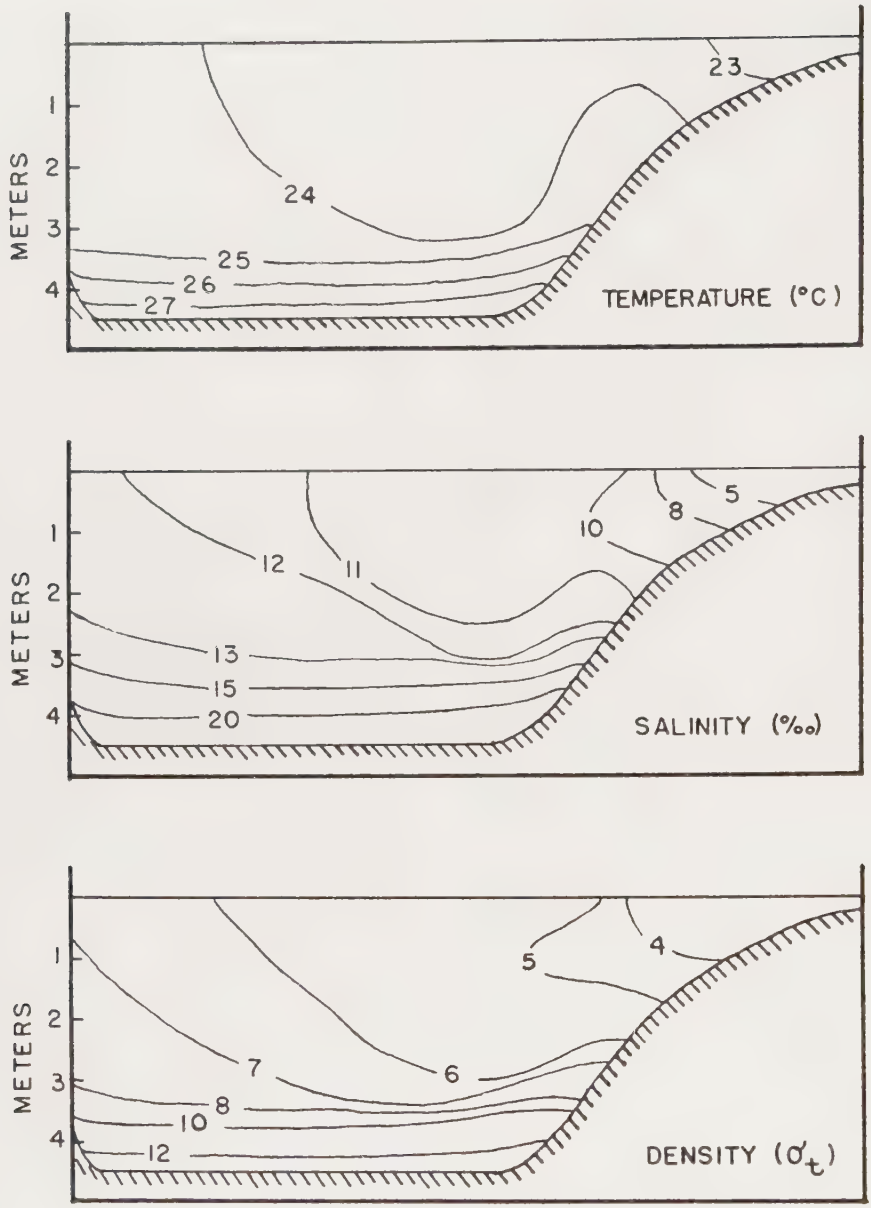

FIG. 2. Temperature, salinity and density distribution in Oyster Pond on August 22, 1958.

plankton upon the growth rate of *Mya*, 40 clams, averaging 17.5 mm in length, were placed in each of five wooden trays filled with sediment. The trays were placed at Stations 1, 2A, 3, 5 and 7 (Fig. 1). At approximately 10-day intervals the clams were measured with calipers to the nearest 0.1 mm. Temperature and salinity were from one-liter samples of water collected daily at each station at the level of the experiment trays.

The plankton population at each station was determined three times each week by analysis of the one-liter sample. A minimum of two Sedgwick-Rafter cell mounts were made from each sample, and the number of flagellated forms was estimated. After addition of formalin and after a 24-hr set-

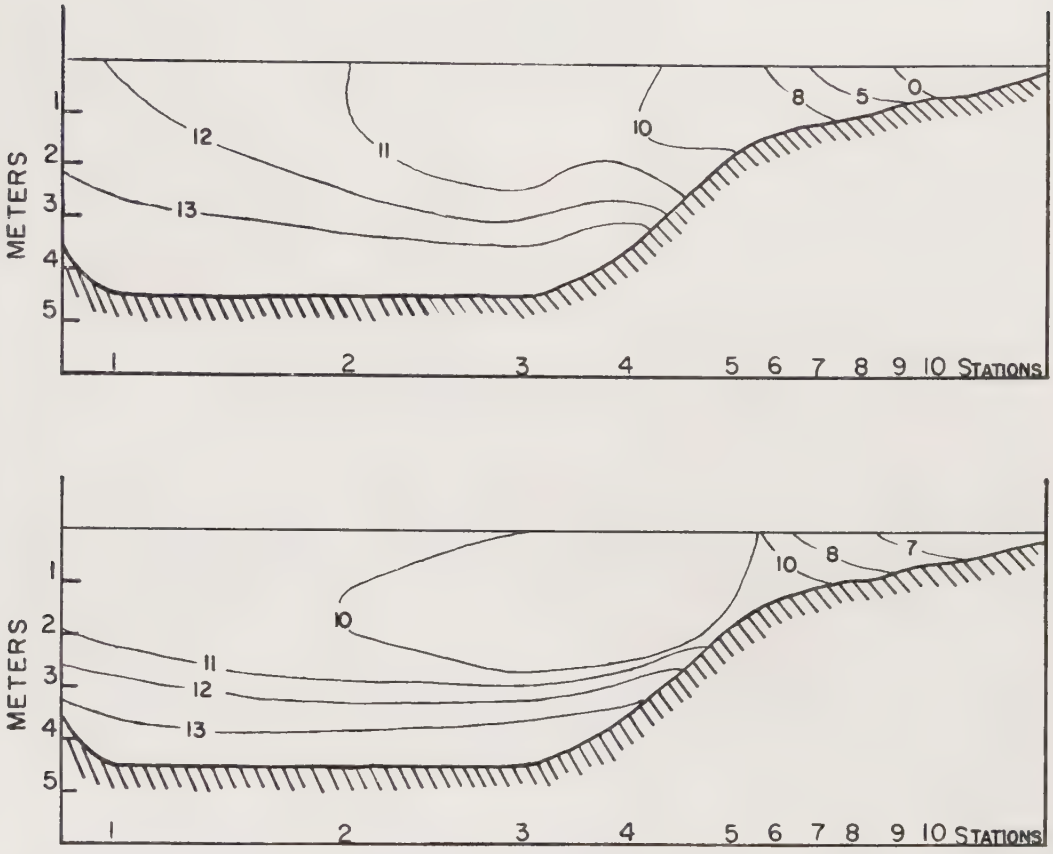

FIG. 3. Salinity distribution in Oyster Pond on August 22, 1958, with wind from the southwest (upper profile), and on August 24, 1958, with wind from the north (lower profile).

tling period, each sample was concentrated to 100 ml by siphoning off the excess liquid with a U-shaped tube. After reshaking, at least two Sedgwick-Rafter cell mounts were made from each, and the nature and abundance of the diatom population was determined.

The samples were then further concentrated to 15 ml, and each was examined under 50 high power microscope fields to give a proportionate estimate by volume of inorganic material, phytoplankton cells, and organic detritus. The remainder of the sample was centrifuged for a 20-min period and the approximate total volume of particulate matter determined.

RESULTS

Certain hydrographic features of Oyster Pond, in particular the salinity distribution during the period when the pond was closed, should be mentioned. Fresh water entering the pond formed a layer over the saline water entrapped by the pond's closing and mixed only very slowly with the subsurface layers. This stability resulted in a positive thermal gradient between surface and bottom layers during the summer (Fig. 2). As Figure 2 also indicates, water of comparatively high salinity was generally confined to the deeper portions of the main basin of the pond.

Northerly winds blew fresh surface water down the upper arm of the pond into the main basin. In compensation, bottom water of high salinity moved into the upper arm, being easily detectable as far as Station 10 within a few hours after a wind shift to the north (Fig. 3). Certain benthic forms such as *Mya* must be able to tolerate ap-

TABLE 1. *Temperature, salinity, and clam growth at Stations 1–10 in Oyster Pond during the period June 17–August 27, 1958*

Station number	Temperature (°C)		Salinity (‰)		Mean weekly growth increments (mm)
	Range	Mean	Range	Mean	
1	16.4–27.0	23.1	9.8–30.5	15.5	1.4
2	17.2–27.4	23.2	10.2–30.0	14.7	1.4
3	19.6–27.0	23.4	8.8–29.0	13.6	1.2
4	15.0–26.9	23.4	7.8–28.8	14.0	1.5
5	19.2–26.9	23.4	5.9–21.0	11.0	1.2
6	18.3–26.8	23.3	3.6–18.5	9.6	1.1
7	16.4–26.6	22.4	1.1–12.8	7.6	1.1
8	15.6–26.9	21.8	0.0–12.0	5.3	0.7
9	12.0–26.2	21.3	0.0–12.0	4.2	0.2
10	11.4–26.2	19.7	0.0–11.8	3.5	0.0

preciable and sudden fluctuations in salinity and temperature as great as the extremes presented in Table 1.

As mentioned previously, Oyster Pond was closed to the sea during the period June 17–August 27; no mortality was recorded among the clams that had been placed in experimental trays at Stations 1–10 during this time. Therefore, although the native population extended no further up the pond than Station 7, clams longer than 12 mm (the smallest clams placed in the trays were 13 mm) were capable of tolerating the salinity that prevailed at Stations 8, 9 and 10 during this period (Table 1). Similarly, clams obtained from an area characterized by constantly high salinities survived as well and grew at the same rate at Stations 4, 6 and 8 as did the native clams at the same stations.

However small *Mya* (2–4 mm in length) were unable to survive above Station 7. Moreover, no clams resulting from the summer spawning succeeded in setting above Station 7, even though it is probable that larvae were carried well above Station 7 in the subsurface layers during the occurrence of northerly winds.

Results from laboratory studies on the ability of *Mya* of different sizes to tolerate immersion in water free of salt for various lengths of time are presented in Figure 4. These results substantiate the evidence obtained from field observations that the larger members of this species are more tolerant to low salinity conditions than the juveniles. The number of days that clams in the 5–10 mm size range can be exposed to various low salinities and resume normal behavior when returned to more favorable conditions are as follows: in 0‰, 2–3 days; in 1‰, 3–4 days; in 2‰, 5–6 days; in 4‰, indefinitely. No clam, regardless of size, appeared capable of survival after immersion in distilled water for a period exceeding 72 hr at about 20°C.

As indicated in Table 1, rate of growth appeared to be directly related to salt concentration within the limits given. As the experiment with Neutral Red suggests (Fig. 5), the pumping rate of *Mya* varies only slightly in water between 15.5–31‰, is sharply reduced when the salinity has dropped to approximately 8‰, and appears to cease altogether at salinities of 4‰ or below. Therefore, even though the water of low salinity at the upper stations may have been deficient in nutritive material, it would seem that the inhibiting effect of low salinities upon the feeding processes satisfactorily explains the different growth rates observed. The fact that growth rate also appears to be directly related to temperature in Table 1 is not considered significant in this case, as further investigations during the fall established that not until the temperature had dropped to 15°C or below did growth rate show a decline from its midsummer value.

The growth rate of clams in experimental

TABLE 2. *Temperature, salinity, and clam growth at Stations 1, 2A, 5 and 7 in Oyster Pond during the period September 18–November 6, 1958*

Station number	Temperature (°C)		Salinity (‰)		Mean weekly growth increments (mm)
	Range	Mean	Range	Mean	
1	14.1–19.8	16.7	15.7–20.7	18.2	1.0
2A	9.5–21.7	14.6	12.0–27.3	16.5	1.0
5	10.1–20.2	14.6	10.8–19.5	14.0	1.5
7	10.0–19.6	14.5	8.2–18.2	13.1	1.5

trays at four stations during the period September 18–November 6 are given in Table 2. During the first week of this experiment, the clams at Station 3 were washed from the tray and lost; those at Station 1 were lost in similar fashion after three weeks.

Contrary to the temperature and salinity distributions, which remained fairly constant during this time, there was a distinct difference in the nature and abundance of phytoplankton in the upper arm of the pond and in the main basin. Diatoms were consistently more numerous at Station 2A, whereas flagellates were considerably more abundant at Station 7 (Table 3). The volume of organic detritus in the water was also found to be greater in the upper arm at Station 7.

DISCUSSION

Turner (1953) states that *Mya arenaria* is fundamentally a creature of the upper estuarine, as opposed to the oceanic, environment. The present results illustrate

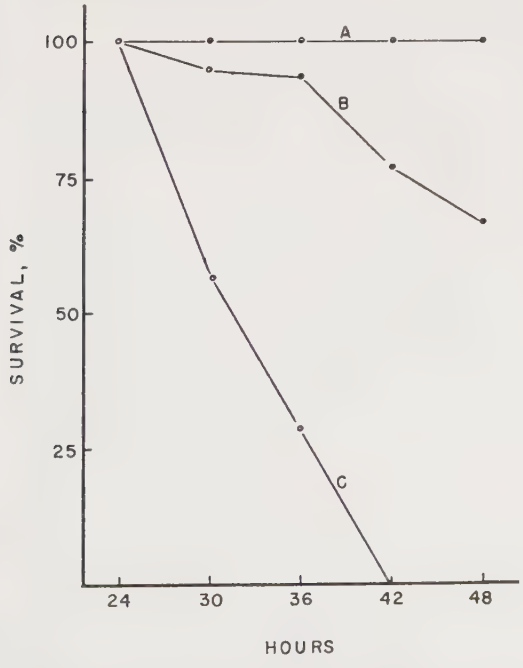

FIG. 4. Percentage of survival of clams of different sizes after immersion in distilled water for given periods of time. A = 15–25 mm; B = 5–10 mm; C = 2–4 mm.

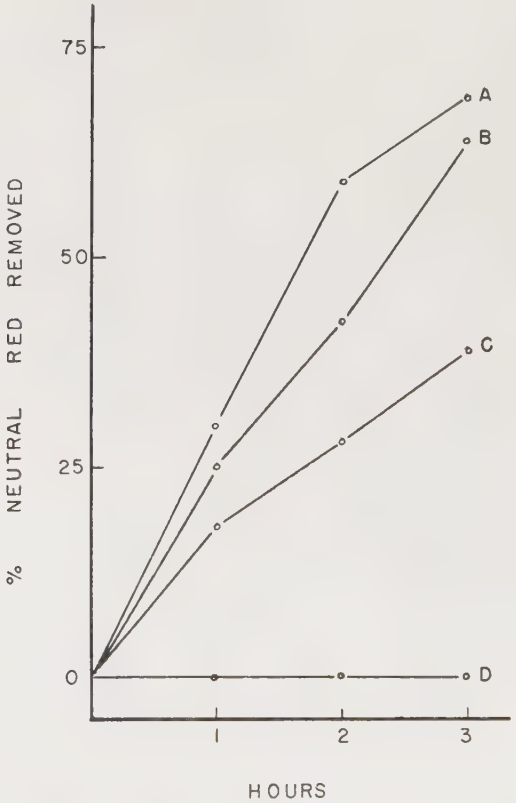

FIG. 5. Relative pumping rates of clams at different salinities. A = 31‰; B = 15.5‰; C = 7.8‰; D = 0–4‰.

TABLE 3. *A comparison of the abundance of diatoms, flagellates, and organic detritus at Stations 2A and 7 in Oyster Pond during the period September 18–November 6, 1958*

	Diatoms Cells/L × 10^6		Flagellates Cells/L × 10^6		Organic detritus cc/L	
	Sta. 2A	Sta. 7	Sta. 2A	Sta. 7	Sta. 2A	Sta. 7
Sept. 18	0.2	0.4	1.9	2.2	0.10	0.35
Sept. 22	1.0	0.5	1.3	3.9	0.30	0.20
Sept. 24	2.1	1.2	1.3	5.8	0.15	0.30
Sept. 26	1.8	1.4	1.7	10.0	0.05	0.35
Sept. 29	1.1	0.9	2.4	6.0	0.05	0.10
Oct. 1	1.0	0.9	1.8	6.5	0.02	0.15
Oct. 3	0.9	0.7	3.2	6.5	0.05	0.05
Oct. 6	0.7	0.6	3.3	5.5	0.02	0.05
Oct. 8	0.9	0.6	2.2	6.8	0.01	0.25
Oct. 10	4.4	2.1	1.8	6.3	0.02	0.10
Oct. 13	1.0	0.4	2.9	6.6	0.10	0.05
Oct. 15	1.0	0.5	2.9	11.6	0.02	0.35
Oct. 17	0.8	0.1	4.5	6.0	0.02	0.05
Oct. 20	0.7	0.2	1.3	2.0	0.05	0.05
Oct. 22	0.4	0.1	1.8	5.7	0.10	0.05
Oct. 24	0.4	0.3	2.2	5.3	0.05	0.20
Oct. 26	0.2	0.2	2.5	3.0	0.05	0.05
Oct. 28	0.3	0.3	4.1	6.0	0.05	0.20
Oct. 30	0.1	0.1	4.6	4.8	0.03	0.15
Nov. 1	0.2	0.1	5.1	5.7	0.02	0.20
Nov. 3	0.1	0.1	4.9	8.2	0.05	0.10
Nov. 5	0.1	0.1	3.4	4.7	0.02	0.03
Mean values	0.9	0.5	2.8	5.9	0.06	0.15

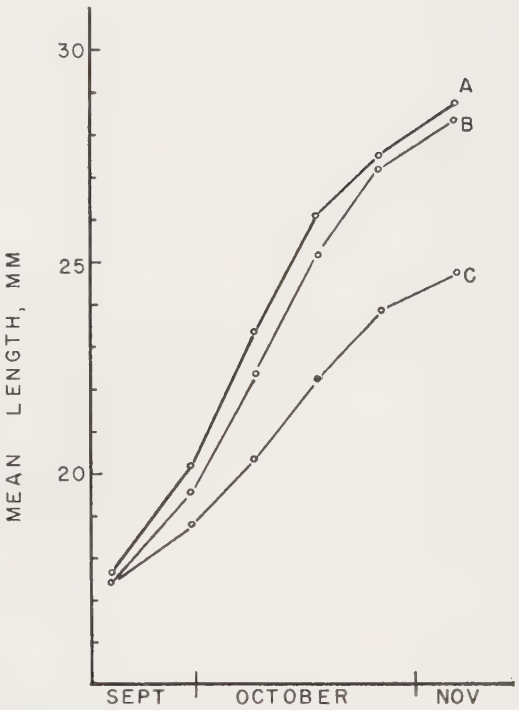

FIG. 6. Comparison of clam growth rates at Stations 2A, 5 and 7 in Oyster Pond during period of September 18 to November 5, 1958. A = Station 7; B = Station 5; C = Station 2A.

again the ability of this species to tolerate sudden and appreciable changes in salinity, a condition common to most estuaries. The fact that the adult clam can withstand sudden salinity fluctuations of an order of 18‰ and apparently can survive for considerable periods of time in salinities as low as 4‰ provides it with a distinct advantage over its less tolerant predators.

With respect to feeding behavior at low salinities, these studies appear to indicate that the salinity may drop as low as 15‰ before the pumping rate is significantly reduced. Moreover, as indicated in Table 1, the weekly growth increment at Station 4 during the summer months averaged 1.5 mm, even though the salinity at this station then averaged only 14‰. This growth rate compares favorably with that determined for a *Mya* population in Quincy Bay during the previous summer, which averaged 1.1 mm per week at a salinity of 28–32‰.

The results also suggest that the greater sensitivity of the juvenile clams will inhibit the establishment of populations in areas subject to sharp fluctuations in salinity. Chanley (1958) found that the young of *Venus mercenaria* were less tolerant to subminimal salt concentrations than adults, and it is probable that the larvae are equally, or perhaps even more, sensitive. It now appears unlikely that the soft clam can successfully reproduce where the salinity averages less than 7‰ and occasionally drops below 1‰, and it is quite possible that its requirements for propagation are even more rigorous.

There are at least two possible reasons why the young of this or any other species of mollusk are more sensitive to low salinities than the adult. Firstly, if Prosser *et al.* (1952) are correct in assuming that death in a hypotonic medium occurs as a result of the breakdown of the enzymatic system, it may be that excessive dilution of the tissues and body fluids is accomplished at a

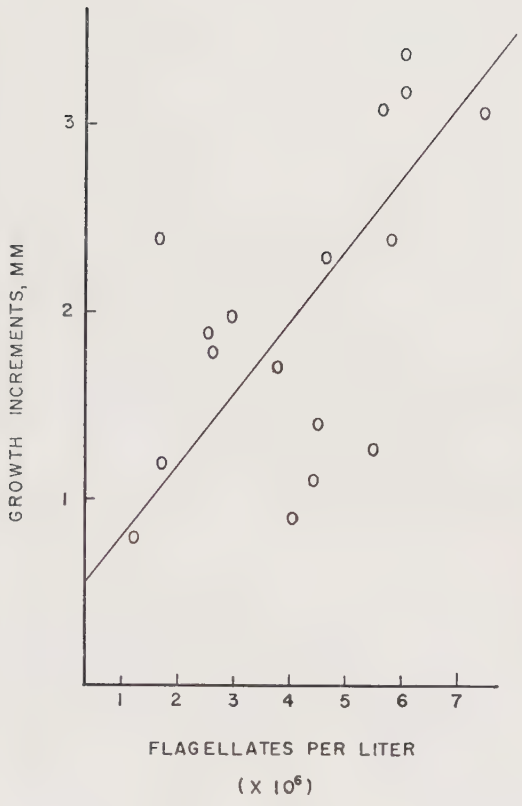

FIG. 7. Increments of clam growth at Stations 1, 2A, 5 and 7 in Oyster Pond, determined at 10-day intervals during fall of 1958, plotted against average flagellate abundance at each station during the corresponding interval.

faster rate in the smaller individuals due to their greater surface area to volume ratio. Secondly, any factor that temporarily interferes with the feeding processes, such as low salinity, may be indirectly lethal to the rapidly developing juvenile but not to the adult. For example, the larvae of *Venus* cultured to the early umbo stage die within 24 hr if food is withheld (Turner and George 1955), whereas the adult may survive for long periods without food.

During the fall the growth of *Mya* was considerably more rapid in the upper arm of the pond where the plankton population was composed almost entirely of flagellates than in the main basin where diatoms comprised a significant portion of the plankton. The growth rates at Stations 2A, 5 and 7 are contrasted in Figure 6. A T-test shows the difference in growth at the upper and lower stations to be significant. Moreover, when the average number of flagellates collected at each station during each of five 10-day growth periods is plotted against mean growth increments at each station for the corresponding periods (Fig. 7), the derived correlation coefficient is significant at the 1% level.

It seems paradoxical that wind-induced mixing was sufficient to destroy the salinity gradient along the axis of the pond but not sufficient to cause homogeneity of phytoplankton. However, an explanation of an analogous situation in Great Pond, Mass., has already been given by Hulburt (1956). It should also be mentioned that a dinoflagellate, believed to be *Kryptoperidinium,* which was abundant in the entering stream water, was often responsible for the larger numbers of flagellates at Station 7 than at Station 2A. Slobodkin (1953) states that the reproductive rate of several species of dinoflagellates is favored by low salinities. The combined effect of these two processes —low reproductive rate among the nonmotile diatoms at the upper stations (Hulburt 1956) and the influx of flagellated forms at the head of the pond—would result in the observed distribution.

The conclusion that flagellates are more readily utilized by clams than are diatoms may appear to conflict with the observations of Pratt and Campbell (1956), who found a negative correlation between flagellate abundance and the growth rate of *Venus mercenaria* but a significant positive correlation between growth and the abundance of diatoms less than 15 μ in size. However, *Venus* occurs most abundantly in the lower estuary (Turner 1953), where diatoms are generally the dominant phytoplanktonic form (Nash 1947; Hulburt 1956). *Mya,* on the other hand, is frequently most abundant in the upper estuary, where flagellates are more likely to outnumber diatoms. The nutritive requirements of the two species, or the relative ability to utilize different phytoplankton, may be correspondingly different.

Organic detritus, also more abundant at

a factor influencing the more rapid growth the upper stations, cannot be dismissed as of the clams there. However, microscopic examination indicated that a fair percentage of it was composed of fragments of crustacean exoskeletons and other refractory substances, of probably no nutritive value to the clam.

REFERENCES

Belding, D. A. 1930. The soft-shelled clam fishery of Massachusetts. Commonwealth of Mass., Dept. of Conservation, Marine Fisheries Series No. 1: 65 pp.

Chanley, P. 1958. Survival of some juvenile bivalves in water of low salinity. Proc. Natl. Shellfish Assoc., **48:** (in press).

Cole, H. A. and B. T. Hepper. 1954. The use of Neutral Red solution for the comparative study of filtration rates of lamellibranchs. Jour. Cons., Int. Explor. Mer, **20:** 197–203.

Hulburt, E. M. 1956. The phytoplankton of Great Pond, Massachusetts. Biol. Bull., **110:** 157–168.

Loosanoff, V. L. 1958. Challenging problems in shellfish biology. *In*: Perspectives in Marine Biology. A symposium held at Scripps Institute of Oceanography, Univ. of Calif., March 24–April 2, 1956. Univ. of Calif. Press.: 483–493.

Nash, C. B. 1947. Environmental characteristics of a river estuary. Chesapeake Biology Laboratory, **64:** 27 pp.

Pratt, D. M. and D. A. Campbell. 1956. Environmental factors affecting growth in *Venus mercenaria.* Limnol. and Oceanogr., **1:** 2–17.

Prosser, C. L., D. W. Bishop, F. A. Brown, T. L. Jahn, and V. J. Wulff. 1952. Comparative Animal Physiology. W. B. Saunders and Co., New York. 888 pp.

Slobodkin, L. B. 1953. A possible initial condition for red tides on the coast of Florida. Jour. Mar. Res., **12:** 148–155.

Turner, H. J. 1951. Fifth Report on Investigations of the Shellfisheries of Massachusetts. Div. Mar. Fish., Dept. of Conservation, Commonwealth of Mass., Boston: 9–10.

———. 1953. A review of the biology of some commercial molluscs of the east coast of North America. Sixth Report on Investigations of the Shellfisheries of Massachusetts. Div. Mar. Fish., Dept. of Conservation, Commonwealth of Mass., Boston: 39–74.

——— and C. J. George. 1955. Some aspects of the behavior of the quahog, *Venus mercenaria,* during the early stages. Eighth Report on Investigations of the Shellfisheries of Massachusetts, Div. Mar. Fish., Dept. of Conservation, Commonwealth of Mass., Boston: 5–14.

UPTAKE OF CESIUM^{137} BY CELLS AND DETRITUS OF *EUGLENA* AND *CHLORELLA*

Louis G. Williams[1]

R. A. Taft Sanitary Engineering Center, Cincinnati 26, Ohio

ABSTRACT

Standard "Euglena" medium putrefied by three common species of bacteria and filtered was found to support unusually heavy blooms of *Euglena* and *Chlorella*. Mass culturing demonstrated that uptake of Cs^{137} by *Euglena* and *Chlorella* is linear with concentrations at all levels that might be encountered in natural aquatic habitats. The rate of uptake of cesium during population growth is greatest in cells of *Euglena* during the stationary period following the exponential growth phase. At this stage the cesium taken in is mostly unbound Following this it is released so that in old cells and detritus it is largely bound cesium. Cesium and potassium behave independently in killed cells of *Chlorella*. Alkali ions, known to be chemically similar, are selected or rejected discriminately by these algae. The presence of antibiotics significantly depresses the uptake of cesium by *Chlorella* and *Euglena*.

INTRODUCTION

The accumulation of cesium^{137} is of interest as a radioactive tracer for an element whose methods of distribution in nature are poorly known. Cesium is chemically similar to potassium and may be expected to enter the solute complex and engage in ionic antagonism, osmosis, permeability regulations, maintenance of colloidal state and similar physiological phenomena. Cs^{137} as a tracer may provide useful information on these processes. Yet the present study emphasizes that important chemical differences exist between this and other alkali ions which make the study of cesium necessary to point out these differences.

Cs^{137} is one of the critical fission products in waste effluents from power reactors and in atomic fallout because of its long half-life of 33 years. Since increasing use of nuclear energy will expose more and more aquatic environments to at least low concentrations of Cs^{137}, information regarding factors controlling the biological fate of radiocesium is urgently needed by the basic and applied sciences which are concerned with isotopes in natural systems.

Algae are noted for their ability to concentrate certain radioisotopes from water. Lackey (1957) found an *Oedogonium* in a settling basin at the Oak Ridge National Laboratory having a count rate of 10,000 times the count rate of the water (37×10^4 /g/min) in which it was growing. Donaldson *et al.* (1956), in a survey of radioactivity in the sea near Bikini, reported the average value for plankton was 71,000 disintegrations/g/min, which was, 7,100 times the average surface value.

Although knowledge of the mechanisms of accumulation of minerals including radioisotopes in plankton and of their action in organisms are still vague, it is significant that certain plankton selectively cumulate specific elements and dissolved organic metabolites (Provasoli 1958) into their bodies from minute amounts in water. In addition to the more common compounds in the protoplasm, cells of phytoplankton cumulate substances which remain in inorganic form and are apparently without metabolic function. In *Valonia*, large coenocytic cells contain 25 to 30 ml of vacuolar sap which is largely inorganic. In this sap potassium has been concentrated 50 fold (Smith 1951). In *Hydrodictyon* potassium may be cumulated as much as 4000 times over the surrounding water (Blinks and Nielsen 1940). Williams and

[1] This is part of an investigation conducted under the Research Participation Program of the Oak Ridge Institute of Nuclear Studies in the Ecology Group, Health Physics Division, Oak Ridge National Laboratory, operated for the U.S. Atomic Energy Commission by the Union Carbide Nuclear Company. The author is now with the R. A. Taft Sanitary Engineering Center, Cincinnati 26, Ohio.

Swanson (1958) report Cs^{137} concentrated 1530 times in *Rhizoclonium hieroglyphicum* (C. Ag.) Küetz.

The concentration of a radionuclide may be accomplished by sorption (adsorption and absorption), which can occur on living or dead material. Radionuclides once concentrated by algae may be further concentrated by ingestion by animals comprising the links of a food chain. Therefore, an important index of the extent of radioactive contamination of aquatic environments is obtained by assay of samples of pond, lake and stream biota. During their growth, living organisms, including all aquatic organisms, take up a wide variety of chemical elements. The extent of uptake varies greatly among species depending on many factors. During this investigation *Chlorella*, with a dead cellulose wall, and *Euglena*, without a cellulose wall but with a living proteinaceous membrane (pellicle), were used for the study of cesium cycling.

The purpose of this investigation was to perform a series of experiments with living *Chlorella* and *Euglena* and so obtain information on the effect of certain factors on the bioaccumulation, exchange and cycling of Cs^{137}. The factors considered included: age of cells, age of culture medium, influence of bacteria and their dissolved products, population density of cells, concentration of stable ions of cesium, influence of elements chemically similar to cesium, position in the growth cycle for the greatest uptake of cesium, retention time of cesium in living cells and their degradation products.

I gratefully acknowledge my indebtedness to Dr. Stanley I. Auerbach, ecologist, Oak Ridge National Laboratory, who made provisions for this study; to H. D. Swanson of the University of Tennessee, who provided technical assistance; and to Dr. and Mrs. James B. Lackey of the University of Florida, who helped to solve some of the problems of culturing.

MATERIALS AND METHODS

Aquaria of 45 L capacity, containing 30 L of nutrient fluid, were used for the mass culturing of algae. Much of the experimental culture work in the past has been done with small volumes in flasks. During this investigation, however, the large volume of fluid had the advantage of minimizing the proportion of cells coming in contact with the sides of the container, and made more working room for taking aliquots. It also yielded a mass more representative of natural conditions, making possible catabolic conditions for bottom cells and an anabolic environment for suspended cells.

Maintenance of cultures.

Stock cultures were maintained in anabolic conditions by transferring the organisms at intervals to fresh nutrient medium. Some of the experimental cultures were maintained for six weeks without refreshing the medium. Experimental cultures of *Euglena intermedia* (Klebs) Schmitz were begun from stocks containing about 48×10^3 cells/ml. Experimental cultures of *Chlorella pyrenoidosa* Chick were started from stock cultures with about 7×10^6 cells /ml. Two large constant temperature rooms were used for this mass culturing. These contained white fluorescent lamps delivering about 380 ft-c, controlled by automatic clocks to produce a 20-hr day and a 4-hr night. The temperature ranged from 27 to 30°C.

Measurement of radioactivity.

Radioactivity was measured by means of a gamma scintillation well-type detector connected to an integrating decade scaler. Glass tubes (10 mm × 75 mm), in which aliquots of the cells were centrifuged, were used in the detector, thereby minimizing losses by transfer. Activities were recorded for whole cultures, supernatants, and cells. As older cultures accumulated metabolic products their cells showed signs of a catabolic balance by settling at the bottom of the aquaria. This necessitated making three readings of activities of the cells as follows: 1) suspended cells, 2) settled cells, and 3) a thoroughly mixed sample of dispersed and bottom cells. The number of cells per ml was determined with a Neubauer hemocytometer.

Preparation of the culture medium.

The nutrient medium was prepared by dilution from a stock nutrient concentrate, which, in turn, was produced by putrefying Starr's (1956) "Euglena" medium (sodium acetate, beef extract, tryptone, yeast extract and soil extract) with *Escherichia coli*, *Flavobacterium aquatile*, and *Proteus vulgaris*. After putrefying at 35°C for 48 hr the bacterial culture was placed in refrigeration for two or more days at 5°C. During refrigeration bacterial action was arrested and particles settled to the bottom. Forty ml of this filtered supernatant when added to 750 ml of water supported healthy blooms of *Euglena* and *Chlorella*. The concentration of potassium in the culture medium, determined by flame photometry, was 12 ppm, unless otherwise indicated.

A preliminary experiment with a bacterial-free culture resulted in abandoning this method of studying mass cesium cycling in algae. However, *Chlorella* and *Euglena* were grown on a limited scale on bacterial-free standard medium containing Cs^{137} chloride. Concentration of Cs^{137} and population density were significantly less than when these algae were grown in the same medium with selected pollution bacteria. Therefore, all the experimental cultures dealing with cesium uptake contained these selected pollution bacteria. During this procedure, *Euglena* received ample supplies of the B-vitamins and other dissolved organic factors known to be essential for its metabolism (Robbins, *et al.* 1956). Growth of *Chlorella* was given a boost by the dissolved organic solids present in the special medium. The algae and pollution bacteria co-dominated the cultures so that during the growth phases the bacteria and algae were mutually beneficial.

However, the forms of bacteria were not wild, since inoculated pollution bacteria grew to the exclusion of other unwanted bacteria. These pollution bacteria are known to compete in the same way in heavily polluted waters in nature.

Euglena and *Chlorella* cultured in standard unputrefied "Euglena" medium did not produce such dense populations as were attained with the putrefied medium rendered bacterial-free by filtration through membrane filters. Bacteria and these algae inoculated into unputrified standard medium resulted in overgrowth by bacteria, which dominated the cultures producing septic conditions during the first few days. This condition was not evident when the processed medium was employed, because enough oxygen from photosynthesis provided aerobic conditions. The tanks were not aerated. Therefore, it appears that the use of this putrefied medium gives the algae an initial bloom advantage not present in unputrefied "Euglena" medium. Furthermore, this method of culturing pollution-tolerant algae is more representative of natural conditions which might help to explain some of the autecological considerations, that may occur downstream from an outfall of organic wastes or sewage.

When cultured in this medium containing Cs^{137}, the radioactivity of the bacteria was about the same as the medium. Nevertheless, the algal cells were always thoroughly washed by centrifugation before measuring their radioactivity.

The concentration factor for Cs^{137}.

The concentration factor used in this report is the ratio of the activity of the wet weight of the washed algal cells (distintegrations/min/g) to the activity of the nutrient medium (disintegrations/min/ml). Cells were separated from the nutrient solution and washed in demineralized water by centrifugation before measuring radioactivity. The water content of mature *Chlorella* and *Euglena* was found to be about 88% for *Euglena* and 90% for *Chlorella* of the wet weight.

RESULTS

Influence of antibiotics on pollution bacteria and uptake of Cs^{137}.

Because mass culturing of algae free of bacteria proved difficult, an experiment was conducted to determine what effect antibiotics might have on reducing the bacterial populations, and what effect this

TABLE 1. *Influence of streptomycin sulfate and penicillin G on bioaccumulation of cesium*^{137} *by* Chlorella *and* Euglena *cultured with common pollution bacteria*

Days from dosing with Cs^{137}	Concentration factor	
	With antibiotics	Without antibiotics
	For *Euglena*	
3	27	51
8	13	178
10	10	481
11	13	564
14	14	706
19	28	625
	For *Chlorella*	
3	48	41
8	48	106
10	51	104
11	47	92
14	55	95
19	53	115

reduction might have on the uptake of Cs^{137} by *Euglena* and *Chlorella*. These algae were cultivated for 19 days in the processed putrefied medium (control) and also in the same medium to which had been added at the outset and at intervals of 72 hr 30 mg of streptomycin sulfate and 5000 units of penicillin G per liter. All of the cultures were dosed with 10 μc per liter of Cs^{137} chloride. There was a marked streptomycin-induced apochlorosis (yellowing) in *Euglena* beginning about the 17th day. Also, cells of *Euglena*, grown in antibiotics, were smaller and had settled on the bottom of the aquaria after the 11th day. The data from this experiment are presented in Table 1.

Apparently the antibiotics reduced the number of cell divisions in *Chlorella*. Those living in the medium without added antibiotics increased to 6.98×10^6 cells/ml. During this same period those cells in the medium containing added antibiotics reached only 2.3×10^6 cells/ml. It is not understood why *Euglena* without added antibiotics showed a concentration factor of 706, while *Euglena* in the same medium (Table 1) with the added bacteriostatic agents was only 13.6. This same phenomenon was exhibited by *Chlorella*, but less marked. *Chlorella*, cultivated without antibiotics, showed a concentration factor of 115 compared to 53 when grown with antibiotics. Perhaps these antibiotics exhibit a direct depressing effect, not only on the bacteria, but to a lesser extent on the algae. However, the action also might be interpreted as indirect to the algae stemming from the reduction of organic factors made available to the algae by bacterial action.

Influence of concentration.

Unialgal cultures of *Euglena* and *Chlorella*, grown in three concentrations of Cs^{137} chloride, demonstrated that the uptake of this radionuclide is linear with concentrations of 1, 5 and 10 μc/L (Figs. 1 and 2). Knauss and Porter (1954) have also found a linear relationship in *Chlorella* for calcium, iron, manganese, zinc, copper and strontium. However, at concentrations of stable cesium of 0.04 mM/L more, the concentration of cesium ceased to be linear; proportionately less cesium was concentrated which reduced the concentration factor.

Morgan and Myers (1950) have reported that uptake of cesium by *Chlorella* is significantly depressed by traces of potassium ion in the exchange solution. An experiment was conducted to test the relative influence of various concentrations of potassium and stable cesium on the uptake of Cs^{137} by *Euglena*. A culture of *Euglena* was distributed equally into several aquaria, and fresh putrefied medium was added so that each had the same number of cells per ml and the same amount of growing solution. After adding various amounts of potassium and stable cesium the cultures were allowed to grow for five days and the concentration factors were computed.

From Fig. 3 one can see that potassium in high concentration had some depressing effect on the uptake of Cs^{137}, but considerably smaller concentrations of stable cesium carrier had greater effect. In the range of concentrations of stable cesium from 0 to 0.15 mM/L, potassium had a slight depressant effect on the uptake of Cs^{137} at tracer levels. When one millimole of potassium was present, increasing the concentration of cesium carrier from 0 to 0.15 mM /L depressed the uptake of tracer cesium.

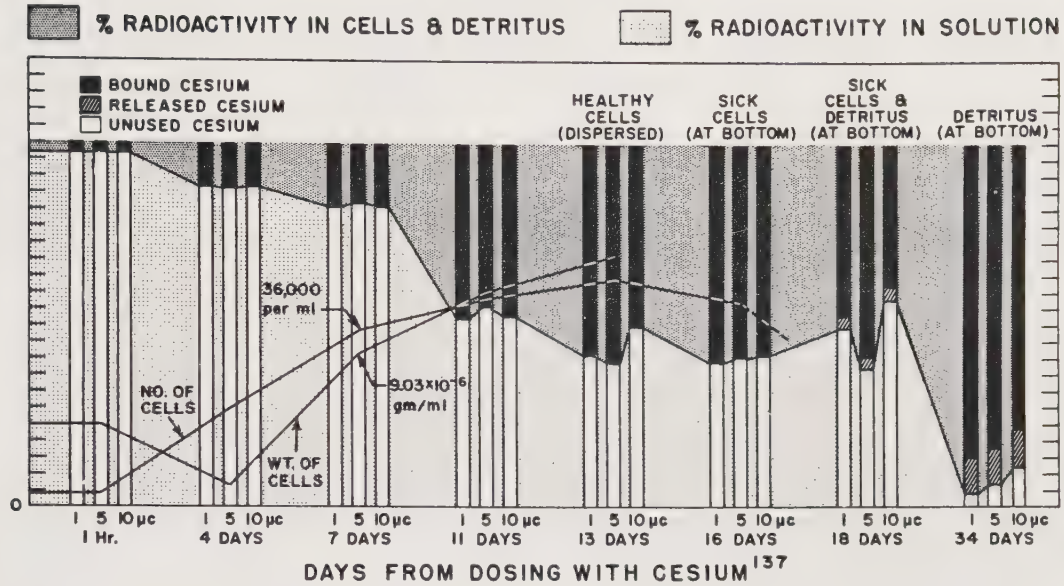

FIG. 1. Uptake of radioactivity from cesium^{137} chloride by cells and detritus from three cultures of *Euglena intermedia*. Weights of cells in g/ml $\times 10^{-6}$ are: at one hour—5.1; 4 days—1.58; 7 days—9.03; 11 days—13.3; 13 days—15.3; 16 days—(with some bottom sick cells)—18.7. Number of cells per ml are: at one hour—800; 4 days—16,000; 16 days (dispersed cells only)—44,000, with catabolic cells on bottom of aquaria. Released cesium was determined by subtracting activity of medium at the surface from the activity of the supernatant of centrifuged cells from the bottom.

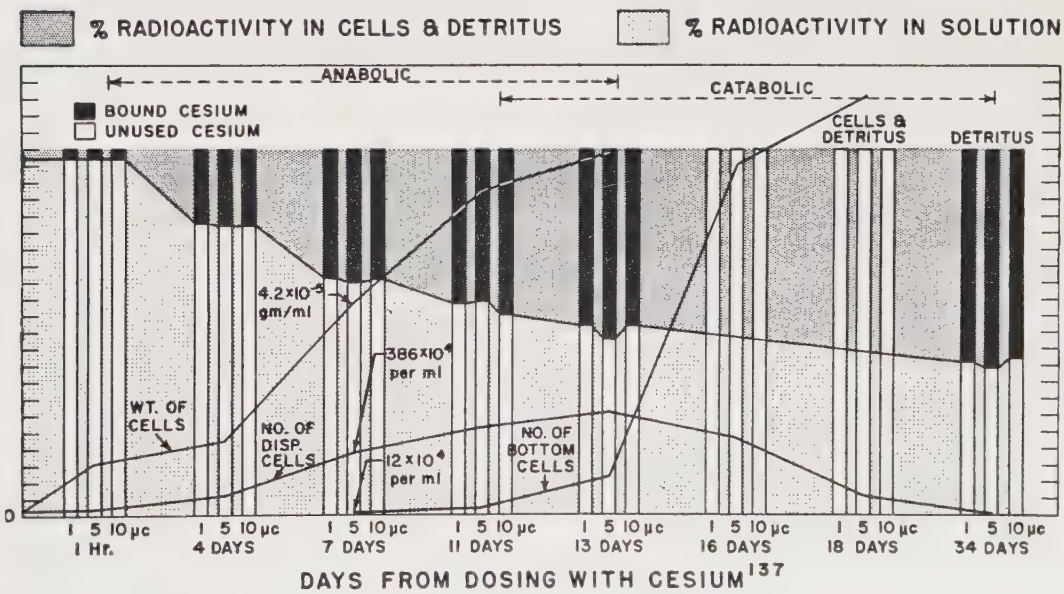

FIG. 2. Uptake of radioactivity from cesium^{137} chloride by cells and detritus of *Chlorella pyrenoidosa*. Weights of dispersed cells in g/ml $\times 10^{-5}$ are: at one hour—0.3; 4 days—3.4; 7 days—4.2; 11 days—6.5; 13 days—7.5. The number of dispersed cells per ml $\times$ 1000 are: 1 hour—380; 4 days—1,128; 7 days—3,860; 11 days—5,365; 13 days—6,250; 16 days—4,716; 18 days—1,018. The radioactivity in solution on the thirty-fourth day was determined by analyzing the activity of a surface aliquot and subtracting this from the activity of the supernatant from bottom cells.

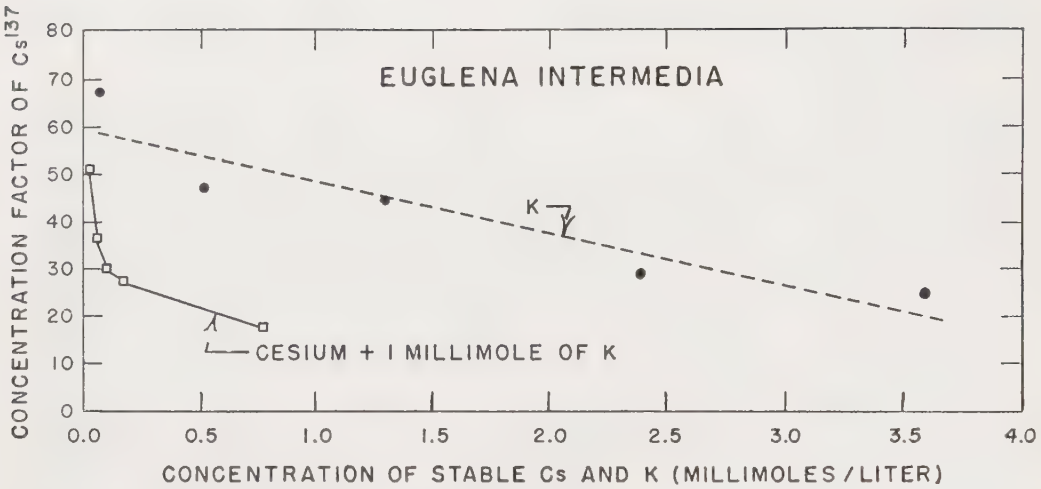

FIG. 3. Influence of potassium and cesium ions in the nutrient medium on the concentration of cesium^{137} by *Euglena intermedia*. The broken line represents the influence of potassium (regression line determined by least squares). The solid line represents the influence of cesium with potassium constant at one millimole per liter.

The further increase of cesium content to 0.75 mM/L caused little additional depression of Cs^{137} uptake. The addition of an equimolar amount of potassium resulted in very little additional depression of the cesium uptake. The effect of potassium in the medium is presumed to be slight, because a concentration factor of 100 was found for *Chlorella* grown in the medium with potassium concentration as high as one millimole per liter.

In experiments during which *Chlorella* was grown in varying concentrations of stable cesium, the concentration factor for Cs^{137} was 46 to 59 in the absence of cesium, 15 to 18 with 0.5 mM/L, and 8 to 12 for other concentrations of stable cesium up to 2.5 mM/L.

The effect of varying ratios of stable cesium to stable potassium on the uptake of Cs^{137} by these algae is shown in Table 2. Live cells took up huge amounts of Cs^{137} even when large amounts of K were present. Killed cells of *Chlorella* took up more Cs^{137} than killed cells of *Euglena*.

Cesium has been reported by MacLeod and Snell (1950) to behave similarly to sodium, potassium and rubidium in the nutrition of lactic acid bacteria. Whether potassium or sodium concentration has any effect on the uptake of cesium by *Chlorella* and *Euglena* was tested by culturing them in the medium containing these ions, and the results are shown in Figs. 4 and 5. The effect of sodium in slightly depressing the uptake of cesium in *Euglena* probably results from alteration of plasma membrane

TABLE 2. *The influence of varying ratios of cesium to postassium on the concentration of cesium*^{137} *in live and formalin-killed cells of* Chlorella *and* Euglena

Ratio of stable cesium to potassium in mM/L			Concentration factor for cesium^{137}
Cs	to	K	
		For *Euglena*	
0		2.4	5 by dead cells
0		2.4	84 live cells
0.62		1.95	22 live cells
1.25		1.55	20 live cells
1.88		1.02	18 live cells
2.50		0.26	17 live cells
2.50		0.26	1 by dead cells
		For *Chlorella*	
0		2.4	36 by dead cells
0		2.4	59 live cells
0.62		1.95	19 live cells
1.25		1.55	12 live cells
1.88		1.02	9 live cells
2.50		0.26	13 live cells
2.50		0.26	2 by dead cells

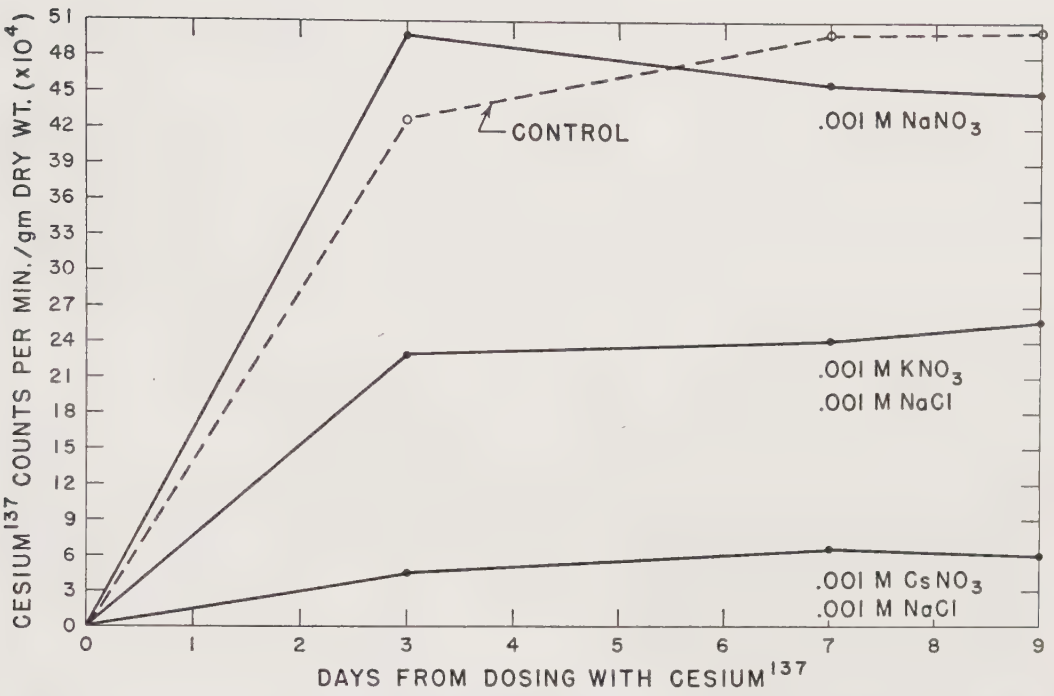

FIG. 4. Influence of stable cesium, potassium, and sodium on the uptake of cesium^{137} by *Chlorella pyrenoidosa*. Each line represents an identical culture, except for the added experimental chemicals.

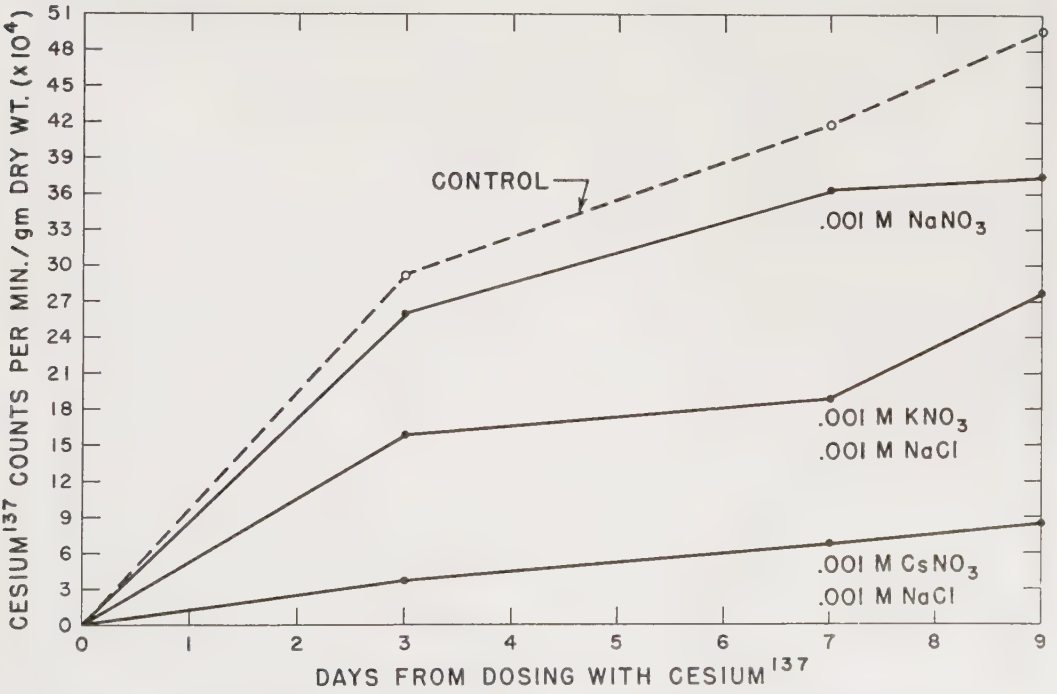

FIG. 5. Influence of stable cesium, potassium, and sodium on the uptake of cesium^{137} by *Euglena intermedia*. Each line represents an identical culture, except for the added experimental chemicals.

permeability by sodium rather than by its antagonistic effect in the metabolic pool. In *Chlorella,* however, sodium showed no effect relative to potassium on the uptake of cesium. In young and dividing *Euglena* and *Chlorella,* potassium enters a metabolic pool with cesium and, therefore, the uptake of cesium is reduced. This, however, does not hold true for old or dead cells.

A potassium-free medium in which these algae would grow was not obtained. However, a putrefied medium was prepared having less than one ppm of potassium. This medium supported a sparse population of *Euglena,* which produced only a few generations by cell division. The addition of one millimole per liter of cesium chloride carrier appeared to produce a slight toxic effect, as the majority of the cells settled to the bottom. However, an addition of one millimole of KCl per liter to this medium supported many generations of cell divisions and a large bloom of healthy *Euglena.* Apparently *Euglena* will take up huge amounts of cesium, but it is unable to substitute cesium for potassium in its metabolism.

The settling of cells on the bottom of aquaria may be associated with changes in illumination and chemicals (nutrients and toxins). Throughout this study the settling of cells was usually associated with changes in the medium which initiated catabolic conditions. An increase in the number of dispersed cells was considered an indication of good growing conditions; conversely, an increase in the population of cells at the bottom marked unhealthy conditions. As the population at the bottom increased significantly, the dispersed population seemed to decrease proportionately. After settling, the cells on the bottom were quiescent for a period before death. By the time bacterial decomposition gave rise to detritus, the number of dispersed cells was less than at the time of inoculation. This catabolic condition might be attributable to cumulation of toxic metabolic wastes. There was, however, one exception noted. Cells of *Euglena,* cultured in solutions containing 0.75 mM of stable cesium or more, concentrated so much cesium that they presumably became toxic or so heavy that they were unable to remain buoyant.

Metabolic cumulation of Cs^{137}.

Rice (1956) demonstrated that dividing cells of *Carteria* took up 64 times as much strontium per cell as nondividing cells. During the present study, healthy dividing cells of *Euglena* cumulated Cs^{137}, but the greatest cumulation appeared to be just following the enlargement phase of cell division (Fig. 1). When cells of *Euglena* from a middle-aged culture were used as an inoculum to fresh culture medium, there was a lag period followed by a phase of exponential growth. During exponential growth the average size of cells was small. The weight of cells increased after the exponential phase of cell division. In Figure 1 the weight of cells can be seen to exceed the exponential cell division curve. Although there is bioaccumulation of Cs^{137} during the exponential growth phase, the greatest uptake by *Euglena* was during the subsequent stationary phase when the cells became heavier. Although this does not follow the usual kind of metabolic uptake by live, rapidly dividing cells, it is believed to be largely metabolic because formalin-killed cells of *Euglena,* at all ages, had small concentration factors. In *Chlorella,* however, the cumulation of cesium per cell appeared to be related to the amount of organic matter produced as follows: 1) the number of cells, 2) size of cells and 3) thickness of cell walls. Young cells are smaller. Young and growing cells have thin walls. Settled cells possess thick walls.

Effect of population density on cesium concentration.

An experiment was designed to investigate the effect of various populations of cells of *Euglena* on the concentration of Cs^{137}. A healthy, dense culture of *Euglena* was concentrated in the centrifuge and then washed by further centrifugation in demineralized water. The washed *Euglena* cells, numbering about 180,000/ml, were resuspended in fresh nutrient medium and distributed to aquaria so that there were

TABLE 3. *The influence of varied populations on the bioaccumulation of cesium*^{137} *by* Euglena *intermedia*

Number of cells/ml		Concentration factor of Cs^{137}		
At beginning	After 40 hr	At 18 hr	At 40 hr	At 96 hr
144,000	146,100	3.4	8.2	62
86,000	113,800	3.4	11.3	58
36,000	60,000	4.6	15.4	28
21,000	68,300	5.9	17.0	26
18,000	55,500	6.4	19.4	19
1,400	27,700	5.3	11.0	16

six fresh population classes varying from 1400 to 144,000 cells/ml. Each of the six cultures was given 10 μc/L of Cs^{137}. Radioactivity measurements were made after 18, 40 and 96 hr. The results are shown in Table 3. Based on uptake of cesium, all of the population classes had the same chronological and physiological age at 18 hr. After 96 hr there were different physiological age classes. The saturated and supersaturated densities were older and took up more cesium. The cells in the sparsely populated cultures were in the exponential phases of growth and took up the least cesium per cell and per weight. This seems to be the situation in most of the experiments dealing with the uptake of cesium. Cells of *Euglena* from low populations, but actively dividing, show relatively low concentration factors. A surge in the uptake of cesium takes place at the time cells cease to divide. Actively dividing cells are relatively light compared to cells that have ceased to divide. Heavy cells continue to sorb more cesium. This might imply that some blooms of algae and their detritus would tend to take up more Cs^{137} in a natural situation three or four days after the establishment of a bloom.

Uptake of cesium by dead algae.

Since it has been shown that dead organic material can have a high affinity for cesium (Williams and Patrick 1957), cells of *Chlorella* and *Euglena,* which had been grown in a medium containing 15 ppm potassium, were washed, killed in formalin and exposed to Cs^{137} chloride. After eight days the concentration factor was 16 for *Euglena* and 418 for *Chlorella.* Because the dead cells of *Chlorella* showed a high concentration factor for cesium, an experiment was performed to test the uptake of cesium from varying levels of cesium, potassium, and a cesium-potassium combination. Healthy living cells of *Chlorella* were washed by centrifugation in demineralized water and killed with formalin. They were then equally distributed into 33 large centrifuge tubes and resuspended in demineralized water. Stable salts were added to the 33 tubes to make three classes of 11 each, as follows: 1) 0 to 5 mM/L of potassium; 2) 0 to 5 mM/L of stable cesium; 3) potassium and cesium in varying ratios but always totaling 2.5 mM/L. Each tube was dosed to give an activity of about one microcurie of Cs^{137} per liter and left standing for 72 hr. Thereupon, the cells in each tube were centrifuged, washed, dried, weighed and analyzed for radioactivity. The dead cells of *Chlorella,* with no added salts and at all levels of potassium 1), were found to concentrate Cs^{137} by a factor varying from 35 to 68; the variations appeared to be random. In mixtures containing stable cesium 2) and 3), the concentration factor varied around one; that is, the algal cell bodies contained the same amount of Cs^{137} as did an equal volume of the medium in which they were suspended.

These results suggest that structural components persist in dead *Chlorella* which adsorb cesium from very dilute solutions and that this adsorption is not affected by the concentration of potassium in the medium.

It may be inferred also that potassium and cesium behave independently on killed cells of *Chlorella.* Membrane permeability controls much of the uptake of cesium during the exponential phase of growth, which may account for its concentration in solutions within the cell. However, as the cells become physiologically older and die, the membranes no longer control the osmosis of cesium. This results in loss of cesium from cell solutions, so that relatively more of it is adsorbed on organic materials. After mass culturing *Euglena* for 34 days, about 83% of the original administered dose of Cs^{137} was bound to cellular detritus or sick

cells; 10%, washed out by centrifugation, was loosely related to cellular materials on the bottom; and 7% remained in solution in the culture medium.

Mraz and Patrick (1957) have shown that vermiculite in the diet of rats preferentially adsorbs cesium from the blood into the gut, thus increasing the amount lost by defecation and correspondingly decreasing the amount lost in the urine. The fact that dead algal protoplasts seem to take up more cesium after they die might indicate that differentially permeable membranes resist the diffusion of cesium into the cells while they are young and healthy and permit more to enter the protoplasts when they become old or die.

Cumulated bound and unbound cesium.

To be able to generalize about cesium cycling in plankton, the concentration of cesium in the cells must be determined at various physiological ages. Some information for *Euglena* and *Chlorella* is given in Figures 1 and 2, and in Table 3.

The sites for holding cesium in and to the cells also are significant in cesium exchange. An experiment was performed to learn something concerning the loci of cesium storage in these algal cells. The cells used for this experiment were mature healthy cells from cultures that had been through the exponential phase and were in the stationary phase of population growth at the time of being dosed with Cs^{137}. Cell densities were about 6×10^6/ml for *Chlorella* and 36×10^3/ml for *Euglena.*

Washed cells of *Chlorella,* having 1,060,000 cpm/g dry wt, were fragmented. These washed fragments showed 645,000 cpm/g dry wt. Cells of *Euglena* having 440,000 cpm/g dry wt when fragmented and washed gave 324,000 cpm/g dry wt. These data imply that much of the cesium is bound in and to the cell solids, while the balance is unbound as free ions or bound to soluble materials in cellular fluids for this particular physiological age grouping.

Data from several of these experiments indicate that most of the cesium is bound to cell wall and particles of cell inclusions in *Chlorella* both dead and alive and at all ages. This is not the situation, however, with *Euglena* where an exchange operates not only between the medium and the cell, but also within the cell between a fluid ionic state and a bound state. Young and dividing cells have low bound cesium, while old cells and cell fragments (detritus) of *Euglena* have relatively high activity from bound cesium. However, the surge in uptake of cesium following the period of exponential growth during the stationary period appears to be mostly an unbound concentration. As the cells become old, much of this inside free cesium becomes bound to cellular particles. Figures 1 and 2 show that much of this radioactivity becomes bound in detritus after 34 days under these conditions.

Compatible nutrient medium, proper illumination, and favorable range of temperature are necessary extrinsic factors in obtaining dense blooms of algae, but the number of cells and the physiological age of cells at the time of inoculation have an important bearing on the time required for blooming and the maximum population attainable. Highest population densities were obtained by maintaining young, enabolic stock cultures. Old stock cultures produce slow blooms, with less than maximum populations, probably because the bacteria are given an initial advantage before the algae begin to co-dominate. When old cultures are used as inocula, a longer period is required to accumulate Cs^{137} from the medium. A larger mass of cells can be obtained in a relatively short time by beginning with actively dividing cells.

By measuring the activity of an aliquot of the culture from which cells and detritus have been removed, it was easy to calculate the per cent of administered dose of Cs^{137} that remains and so determine the extent to which these algae decontaminate their nutrient medium. During this investigation the highest decontamination was 93% in 34 days by *Euglena* and its detritus. *Chlorella* decontaminated the medium by about 47% at several levels of concentration of Cs^{137} in about 13 days.

During the early stages of growth, a sample of cells from the top and one from

the bottom of an aquarium have the same radioactivity. However, as cells settle on the bottom, more and more activity is taken out of the solution and delivered to the bottom as bound cesium in settled old cells and later as detritus. This results in a condition in which the bottom sample is many times more radioactive than a surface sample.

In natural aquatic habitats, small concentrations of radioactive cesium might be expected to be cycled out of solution and carried to the bottom by algal blooms, producing "hot" conditions for bottom-dwelling organisms. After the radioactive cells settle on the bottom, they die and give rise to "hot" detritus. Thus cesium in the aqueous phase has been converted into a solid or bound phase in the algal detritus.

REFERENCES

BLINKS, L. R., AND J. P. NIELSEN. 1940. The cell sap of *Hydrodictyon*. J. Gen. Physiol., **23**: 551–559.

DONALDSON, LAUREN R., A. H. SEYMOUR, E. E. HELD, N. O. HINES, F. G. LOWMAN, P. R. OLSON, AND A. D. WILANDER. 1956. Survey of radioactivity in the sea near Bikini and Eniwetok atolls, June 11–24. Applied Fisheries Lab., Univ. of Wash., Seattle, Wash.

KNAUSS, H. J., AND J. W. PORTER. 1954. The absorption of inorganic ions by *Chlorella pyrenoidosa*. Plant Physiol., **29**: 229–234.

LACKEY, JAMES B. 1957. The suspended microbiota of the Clinch River and adjacent waters in relation to radioactivity in the summer of 1956. Oak Ridge National Laboratory Report No. 2410. *Reprinted as*: Tech. Paper 145, Florida Engineering and Indus. Exp. Sta. 1958.

MACLEOD, R. A., AND E. E. SNELL. 1950. The relation of ion antagonism to inorganic nutrition of lactic acid bacteria. J. Bacteriology. **59**: 783–792.

MORGAN, L. O., AND J. MYERS. 1950. Biological accumulation of inorganic materials by algae. Final report to the Office of Naval Research. Univ. of Texas.

MRAZ, FRANK R., AND HOMER PATRICK. 1957. Factors influencing excretory pattern of cesium^{134}, potassium^{42}, and rubidium^{86} in rats. Pro. Soc. Exp. Biol. Med., **94**: 409–412.

PROVASOLI, LUIGI. 1958. Nutrition and ecology of protozoa and algae. Annual Rev. Microb., **12**: 279–308.

———, S. H. HUTNER AND IRMA J. PINTNER. 1951. Destruction of chloroplasts by streptomycin. Cold Spring Harbor Symposia. **16**: 113–120.

RICE, T. R. 1956. The accumulation and exchange of strontium by marine planktonic algae. Limnol. Oceanogr., **1**: 123–138.

ROBBINS, WILLIAM J., ANNETTE HERVEY AND MARY E. STEBBENS. 1956. *Euglena* and vitamin B_{12}. Annals New York Acad. Sci., **56**: 818–829.

SMITH, GILBERT M. 1951. Manual of Phycology. Chronica Botanica Co., Waltham, Mass.

STARR, R. C. 1956. Culture collection of algae at Indiana University. Lloydia, **19**: 129–149.

WILLIAMS, LOUIS G., AND H. D. SWANSON. 1958. Concentration of cesium^{137} by algae. Science, **127**: 187–188.

———, AND H. PATRICK. 1957. Metabolism of cesium^{134} in rabbits. Arch. Biochem. Biophys., **70**: 464–468.

THE ACCUMULATION OF NITROSYL RUTHENIUM BY FINE PARTICLES AND MARINE ORGANISMS

Raymond F. Jones[1]

United Kingdom Atomic Energy Authority, Industrial Group, Research and Development Branch, Windscale, Cumberland, England

ABSTRACT

The accumulation of nitrosyl ruthenium^{106} by fine particles, algae and animals from sea water is described. The presence of ferric hydroxide on the sand and silt particles enhances Ru^{106} accumulation. Uptake of Ru^{106} by marine algae is a surface phenomenon associated with the extra-cellular polysaccharide material. Fish accumulate the nuclide principally in the gut, gills and skin. In the case of mussels, the shell accounts for the major fraction of accumulated radioactivity. Nitrosyl Ru^{106} species present in the radioactive effluent normally discharged to sea from Windscale Works are compared to nitrosyl Ru^{106} of non-factory origin by paper chromatography. The addition of nitrosyl ruthenium trinitrate to sea water results in the formation of nitrosyl ruthenium chloride which is adsorbed to a greater extent than the Ru species present in the factory effluents.

INTRODUCTION

During the irradiation of uranium fuels in nuclear reactors for the production of plutonium for military purposes, or nuclear power for the generation of electricity, a number of radioactive fission products are formed. In general, these fission product wastes, when concentrated, are of such high radioactivity that only storage in special containers or in isolated regions can be considered. In addition, there arise substantial quantities of waste in which the radioactivity is too dilute to warrant economical concentration to a form suitable for storage. For coastal countries and islands disposal of this low-level radioactive waste into the sea is, however, practicable (Saddington and Templeton 1958; Dunster and Farmer 1958).

At the United Kingdom Atomic Energy Authority's Windscale Works, Sellafield, Cumberland, irradiated uranium rods are decanned and dissolved in nitric acid, and the uranium and plutonium separated from the fission products. During the first stage of this separation process most of the fission products are removed and the highly radioactive liquor is stored permanently in shielded stainless steel tanks. Further separation of the uranium and plutonium by aqueous and organic solvents gives rise to aqueous streams containing fission products which have failed to be removed in the primary separation. These liquids are low in radioactivity and, following a period of storage for a year or so to allow for further radioactive decay, are subjected to effluent treatment and discharged into the sea through a mild steel pipeline.

Experimental discharges of radioactive effluent have been made from the Windscale Works to the coastal water off Cumberland since 1952. Dunster and Farmer (1958) have reviewed this experimental discharge program and pointed out that by extensive monitoring of the marine environment, particularly of fish, seaweed, sea bed and shore sand, it is possible to establish the maximum permissible discharge rate and to assess the maximum permissible levels for the various types of marine samples. These authors state that some 15,000 c of fission products can be released safely into the sea off the Cumberland coast each month.

One of the major fission products in this radioactive effluent is Ru^{106}. Ruthenium compounds arising from the nitric acid treatment of the irradiated uranium are considered to be mainly nitrosyl-nitrato and nitrosyl-nitro ruthenium complexes (Fletcher *et al.* 1955).

[1]Present address: Department of Biology, Princeton University, Princeton, New Jersey.

The object of the present work has been to study the behavior of pure nitrosyl-nitrato Ru^{106} in sea water, its accumulation by fine particles and marine organisms and to compare it with the nitrosyl Ru^{106} complexes occurring in two factory effluent liquors which normally undergo treatment and discharge to sea. One of these, MAL 4, is the medium active liquor arising from the plutonium separation process, the other, MAL 6, is the medium active liquor from the uranium purification process.

The author is indebted to the Managing Director of the Industrial Group of the United Kingdom Atomic Energy Authority for permission to publish this material.

MATERIALS AND METHODS

Nitrosyl ruthenium trinitrate in HNO_3 (9N) was obtained from The Radiochemical Centre, Amersham, England. The radioactive aqueous raffinates MAL 4 and MAL 6 were obtained from the storage compounds at Windscale Works where they had been stored for just over one year. Of the total radioactivity of the fission products in MAL 4 and MAL 6, Ru^{106} and Ru^{103} together accounted for some 96 to 98% of the total; Ru^{106} contributing 90 to 92%. These aqueous raffinates were neutralized with Na_2CO_3 (10%) to pH 7.0, and after 24 hr were filtered through Whatman 54 paper and the filtrates made up to a known volume with sea water. These solutions were therefore comparable with those discharged into the sea through the effluent pipe.

Unless otherwise stated all solutions of nitrosyl ruthenium were in sea water of pH 7.6 to 7.9. In experiments where a direct comparison was made between the behavior of MAL 4, MAL 6 and the Amersham nitrosyl ruthenium, solutions of the same specific Ru^{106} radioactivity were employed. All solutions were allowed to equilibrate with sea water for three days before use.

The specific gravity of sea bed material from off the Cumberland coast, as determined with bromoform, was found to lie between 2.57 and 2.62 for particles within the size range 1 to 300μ. With such a small variation in density it was possible to fractionate the material into groups of definite particle size by standard gravitational sedimentation methods. Two major size groups were obtained: one with particles of diameters ranging from 200 to 300μ designated sand, the other with particles varying in size from 1 to 50μ in diameter designated silt. For uptake experiments non-radioactive sand and silt particles were used. These were collected from the Cumberland coast remote from Sellafield, washed in filtered sea water and air-dried before use. Accurately weighed samples of these particles of pre-determined diameter were placed in 50 ml conical flasks to which 25 ml of nitrosyl Ru^{106} in sea water was added. Each flask was stoppered and shaken at constant speed on a Microid flask shaker, for various periods of time. The content of each flask was centrifuged to remove the particles and 0.5 ml aliquots of the supernatant assayed for radioactivity as described below. Control samples (Ru^{106}–sea water without particles) were similarly treated. All analyses were carried out in duplicate. The difference in radioactivity between the sample supernatant and that of the control was taken to represent the nitrosyl Ru^{106} accumulated by the particles.

Marine algae, *Porphyra laciniata*, *Ulva lactuca* and *Laminaria digitata*, collected from intertidal zones remote from the Sellafield area, were placed in filtered sea water for 2 days and well washed to remove adhering sand and silt particles. Each sample was then placed in a 50 L glass tank containing 30 L of Ru^{106}–sea water which had previously been equilibrated for 3 days. The tanks were aerated, kept at $10 \pm 2°C$, and illuminated at 70 lux for 8 hr each day. Under these conditions the algae remained in a healthy condition for some 4 weeks.

Where discs of seaweed were used for experimental purposes, a No. 11 cork borer cutting discs of 1.6 cm diameter was employed. Before uptake experiments, the discs were well washed in aeriated sea water for 24 hr. In all such experiments, 5 discs were placed in each 50 ml beaker which contained 20 ml of Ru^{106}–sea water.

For studies on the accumulation of nitrosyl ruthenium by micro-organisms, the marine diatom *Phaeodactylum tricornutum* was obtained from the Plymouth Marine Laboratory. The micro-organism was cultured in an enriched sea water medium (Ketchum and Redfield 1938) and illuminated at 500 lux for 12 hr per day.

Plaice, *Pleuronectes platessa,* 20 cm long and approximately 3 years old, were caught by trawl some 2 to 3 miles off shore from Whitehaven and transported to the laboratory in aerated sea water. The fish were placed in stock tanks of filtered sea water for 2 weeks without food to ensure complete clearing of their gut contents. They were then fed on species of the marine annelid *Nereis.* For uptake experiments, individual fish were kept in 50 L glass tanks containing the radioactive isotope in 30 L of sea water of aerated sea water. For analysis, each fish was anaesthetized by flooding the operculum with ether and the various organs were dissected, care being taken to prevent cross contamination of tissues.

Marine mussels, *Mytilus edulis,* were supplied by the Plymouth Marine Laboratory. Mussels of similar size were used throughout, and before use all attached organisms were removed from their shells.

All samples, whether seaweed, fish, mussels or sedimentary particles, were dried for 24 hr in an electric oven at 110°C and then dry ashed in a muffle furnace at 480°C for 16 hr. Weighed samples of ash (10 to 100 mg) were spread on aluminum trays and the β activity counted under the 2.3 mg/cm^2 window of an EHM 2 tube for not less than 3,000 counts.

Radioactive sea water samples of 0.5 ml were pipetted onto aluminum trays, dried under an infra red lamp and β counted as above. A standard source of nitrosyl Ru^{106} in sea water was dried on an aluminum tray and used to estimate the counter efficiency. Ru^{106} in MAL 4 and MAL 6 solutions was estimated by counting the samples and the standard Ru^{106} source through a 50 mg/cm^2 aluminum absorber.

For examination of the nitrosyl ruthenium species, the method employed by Wain, Brown and Fletcher (1957) using Whatman CRL/1 chromatograph paper was used with slight modification. The solvent used for elution was methyl isopropyl ketone equilibrated with HNO_3 (3M). The individual paper strips were divided into 18 sections of 0.5 cm and 1 μL of the solution applied to the center of section 1. The paper was folded into a cylinder with the aid of three strips of cellotape, and the ascending method of elution carried out in a 600 ml beaker using a petri dish as a lid to prevent evaporation of the solvent. Elution was allowed to take place until the solvent front reached section 18. At 20°C this took approximately 30 min. The chromatograms were removed and dried in an electric oven at 80°C for 2 to 3 min. Each strip was cut out and the individual sections cut and mounted with cellotape to aluminum counting trays. The Ru^{106} present was estimated by counting the activity of the paper strips and a similar standard Ru^{106} source under constant geometry. Histograms were plotted to show the distribution of the nitrosyl ruthenium species.

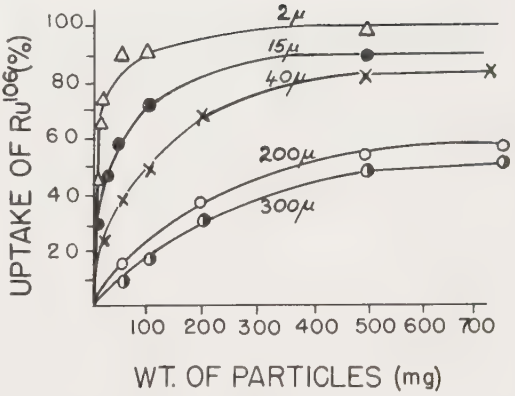

FIG. 1. Uptake of nitrosyl ruthenium by particles of sand and silt from sea water.

RESULTS

Uptake of nitrosyl ruthenium by marine sedimentary particles.

The uptake of Ru^{106} by particles of marine sand and silt of various diameters, after being shaken for 6 hr in sea water containing the same specific activity of Amersham nitrosyl Ru^{106} is shown in Figure 1. The

TABLE 1. *Distribution of radioactivity in sea bed material collected near pipeline outlet, Sellafield (November 1956)*

Particle size microns	Quantity % by wt.		Radioactivity % of total		Radioactivity $\mu c/g$ dry wt.
<4	4.0		20.3		6.2×10^{-3}
4–10	6.1	= 24.1	22.9	= 72.2	4.6×10^{-3}
10–20	14.0		29.0		2.5×10^{-3}
20–50	22.6		16.8		9.0×10^{-4}
50–100	27.4	= 76.0	6.8	= 27.9	3.0×10^{-4}
100–200	26.0		4.3		2.0×10^{-4}

amount of Ru^{106} accumulated increases with decreasing particle size. If the surface areas of the particles are calculated as spherical, then the uptake of the nitrosyl Ru^{106} is proportional to those areas (Fig. 2).

The upper 0.5 to 1.0 in of sediments from the vicinity of the pipeline outlet at Windscale comprises 75% by weight of particles greater than 20μ in diameter. The remaining 25% which are less than 20μ in diameter, however, account for some 70% of the total radioactivity of the sea bed (Table 1). Nevertheless, the radioactivity per unit surface area was similar regardless of particle size (Table 2). The distribution of radioactivity associated with this material was found to be 70 to 80% as Ru^{106} and Ru^{103}, 10 to 20% as Ce^{144}, 5 to 10% rare earths, 3 to 4% as Zr^{95} + Nb^{95} and less than 1% for Sr^{90}.

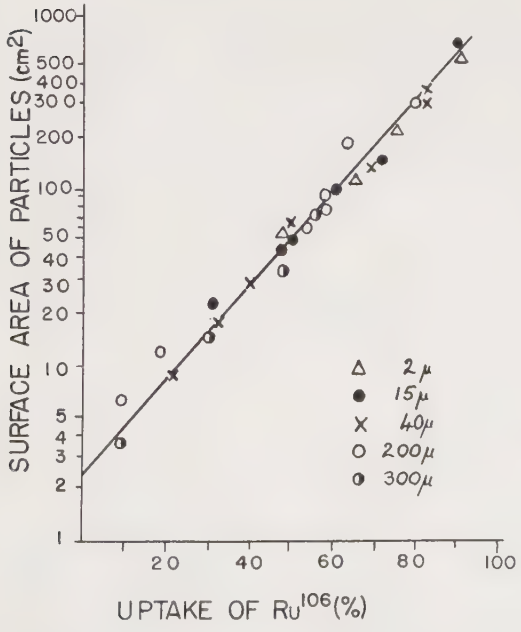

FIG. 2. Uptake of nitrosyl ruthenium by particles of different diameter.

The uptake of Ru^{106} by sand, from sea water containing varying concentrations of the Amersham nitrosyl Ru^{106} follows an adsorption isotherm, (Fig. 3) which is similar to that described by Freundlich:

$$\frac{x}{m} = aC^b \quad \text{or} \quad \log\frac{x}{m} = \log a + b \log C$$

where x is the mass of solute adsorbed, m is the mass of adsorbent, C is the final concentration of the solute in the continuous phase, and a and b are constants. The accumulation of Ru^{106} by sand particles from sea water containing MAL 4 or MAL 6 filtrates, or Amersham nitrosyl ruthenium of the same specific activity, is shown in Figure 4. The uptake of the Amersham form is greater by a factor of 5. Neutralization of the Amersham ruthenium with Na_2CO_3 before addition to sea water results in even greater uptake.

TABLE 2. *Radioactivity per unit surface area of particles from sea bed material collected near pipeline outlet, Sellafield (November 1956)*

Particle size microns	Radioactivity $\mu c/cm^2$
4.0	1.0×10^{-6}
8.0	0.9×10^{-6}
10.0	0.9×10^{-6}
14.0	1.1×10^{-6}
40.0	1.2×10^{-6}
90.0	2.2×10^{-6}
200.0	1.8×10^{-6}

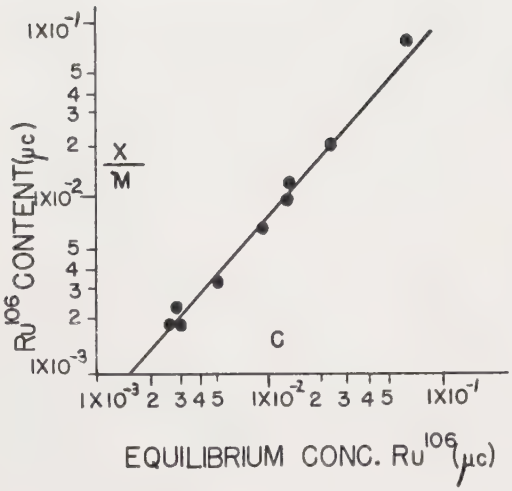

FIG. 3. Adsorption isotherm for nitrosyl ruthenium and sand particles.

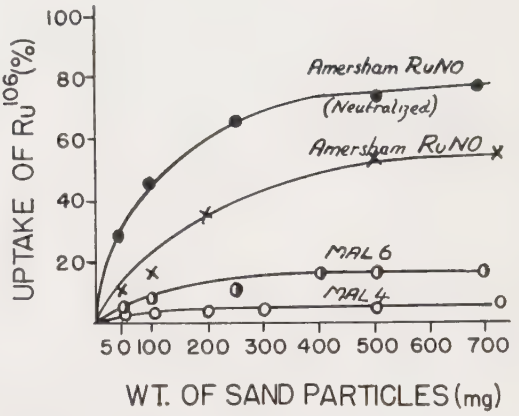

FIG. 4. Uptake of nitrosyl ruthenium of various origins by sand particles.

The Ru^{106} was not easily removed from the marine sedimentary particles. Less than 7% of the Ru^{106} activity was removed by washing in sea water or in sea water at pH 2, 4 or 6. However, 50% nitric acid or a 5% solution of potassium hydroxide and potassium periodate removed 80 to 90%. It appears therefore, that although uptake follows an adsorption isotherm, chemical forces bind the ruthenium to the surface of the particles. The role of ferric hydroxide in such a process was therefore investigated.

The role of ferric hydroxide

The addition of a ferric salt to sea water results in hydrolysis of the ferric salt to form ferric hydroxide, which flocculates and sediments. Where rivers flow to the sea via iron ore deposits, iron is brought to the sea in relatively large quantities, particularly as colloidal particles. These conditions hold for the water off the Cumberland coast. The concentrations of iron in offshore water varies from 0.01 to 0.05 mg Fe/L. Inshore waters, however, are considerably higher in their iron content. In the vicinity of the pipeline end at Sellafield, values of 0.4 to 0.8 mg Fe/L have been determined. When ferric ions are added to sea water containing Amersham nitrosyl Ru^{106} and shaken for 6 hr, there results an insoluble complex between the iron and the ruthenium which can be removed by centrifugation. In such an experiment where the concentration of the Ru^{106} was $2.5 \times 10^{-3}\mu c$/ml, some 70% of the Ru^{106} was removed at an iron concentration of 1 ppm (Fig. 5).

That a similar complexing is likely to occur between the nitrosyl Ru^{106} and the ferric layer present on the surface of sand and silt particles is seen in Figure 6. Removal of the ferric hydroxide layers by treatment with hot HCl (N), followed by washing with sea water, reduced the adsorption of Ru^{106}. Similar sized sand particles were treated with HCl in the same way, but, following the sea water wash they were shaken with ferric chloride solutions of different concentration for 24 hr.

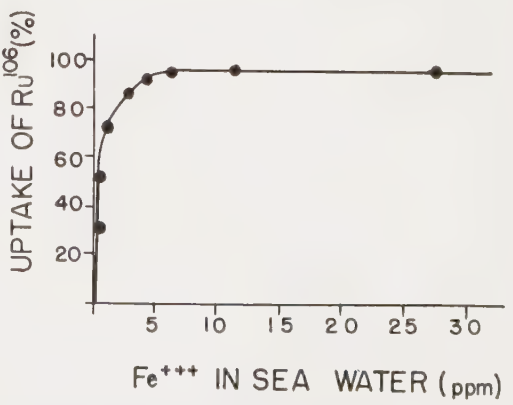

FIG. 5. Effect of ferric ions in sea water upon the precipitation of nitrosyl ruthenium.

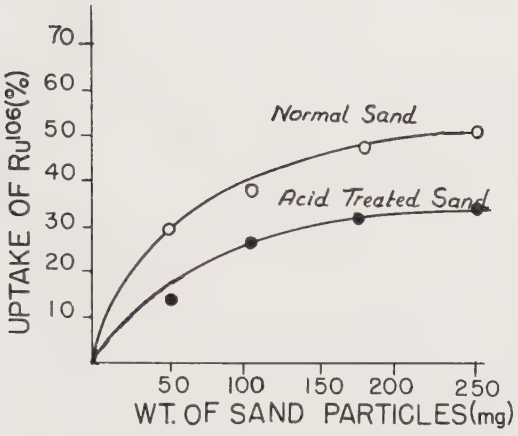

FIG. 6. Uptake of nitrosyl ruthenium by normal shore sand, and shore sand after HCl treatment.

They were then removed, well washed with sea water, and the uptake of Amersham nitrosyl Ru^{106} from sea water measured, together with the amount of iron present on the sand. Figure 7 shows that, by approximately doubling the concentration of iron on the sand particles, the uptake of the Ru^{106} is similarly increased.

Uptake of nitrosyl ruthenium by marine organisms.

Three species of algae, *Porphyra laciniata*, *Ulva lactuca* and *Laminaria digitata*, which were free from fission product contamination, were placed in a tank of sea water containing Amersham nitrosyl Ru^{106}, and at

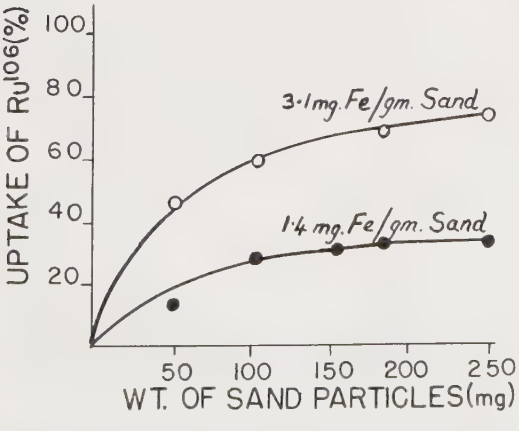

FIG. 7. Uptake of nitrosyl ruthenium by Fe contaminated sand.

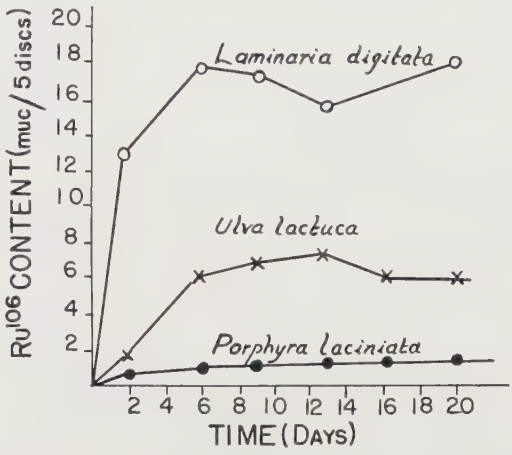

FIG. 8. Uptake of nitrosyl ruthenium by three species of marine algae.

various intervals of time, discs of the thalli were removed and analyzed for Ru^{106} con- 0.08% reduced the amount of Ru^{106} adtent. Figure 8 indicates that the brown alga, *Laminaria digitata*, accumulates more Ru^{106} per unit surface area than the other two species. Evidence that the different chemical composition of the cell surface of these algae may account for the difference was obtained by extracting alginic acid from the non-radioactive brown algae, *Fucus vesiculosus* and *L. digitata*, and studying its ability to complex with Ru^{106}. Alginic acid, both as the free acid and the sodium salt was found to combine with Ru^{106} to form a soluble complex. In an experiment on the uptake of Ru^{106} by sand in calcium-free sea water, the presence of sodium alginate in concentrations between 0.02 and sorbed to the sand by some 70 to 80%. Calcium-free sea water was used to prevent the precipitation of calcium alginate.

The extra-cellular space in *P. laciniata*, as determined by the amount of phenol red which passed into the living cells compared to killed cells (by the method of Eppley and Blinks 1957), accounts for some 40% of the total space available. Figure 9, however, shows the uptake of Amersham nitrosyl Ru^{106} by living and killed discs of *Porphyra* is about the same. Since the Ru^{106} does not enter the cells of the alga, a surface phenomenon, would appear to account for the

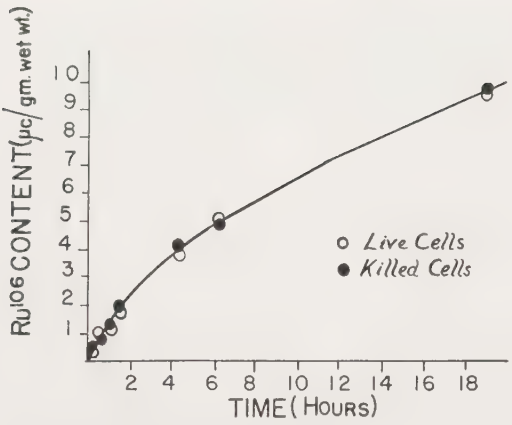

FIG. 9. Uptake of nitrosyl ruthenium by living and killed discs of *Porphyra laciniata*.

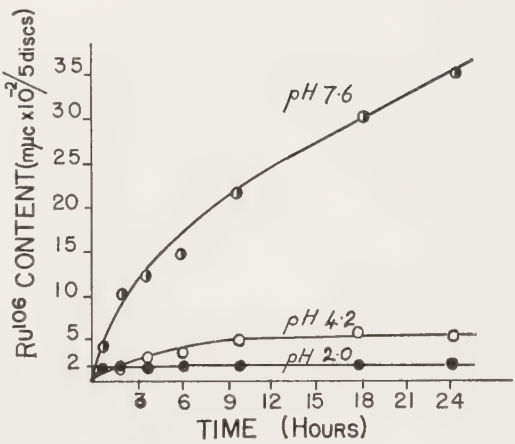

FIG. 11. Effect of pH on uptake of nitrosyl ruthenium by *Porphyra laciniata*.

accumulation. In fact, the uptake of Amersham nitrosyl Ru^{106} by discs of *Porphyra*, immersed in sea water of different Ru^{106} content for periods of 1, 6 and 24 hr, is in agreement with Freundlich's adsorption isotherm (Fig. 10). If the contaminated *Porphyra* is placed in sea water free of Ru^{106}, the weed loses 60% of its radioactivity in 1 hr, showing that desorption occurs and that the binding is not as strong as to the particles of sand and silt. The uptake of Amersham nitrosyl Ru^{106} by *Porphyra* is pH dependent (Fig. 11), and therefore cation-exchange reactions may also occur at the algal surface.

The accumulation of Ru^{106}, by *Porphyra*, from MAL 4 and MAL 6 in sea water compared with that of the Amersham form in sea water, is shown in Figure 12. The MAL 4 and MAL 6 forms of Ru^{106} are not accumulated to any great extent, as compared with the Amersham nitrosyl Ru^{106}.

Some studies on the accumulation of Amersham nitrosyl Ru^{106} from sea water by the marine diatom, *Phaeodactylum tricornutum*, show that this organism is as effective as fine particles of sand in removing Ru^{106} from solution in sea water. No significant difference in mode of uptake oc-

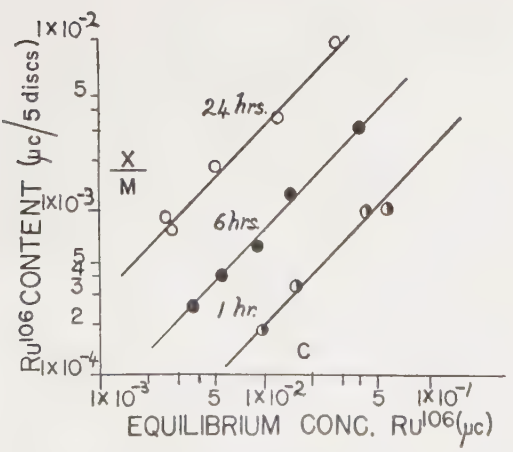

FIG. 10. Adsorption isotherms for nitrosyl ruthenium and *Porphyra laciniata*.

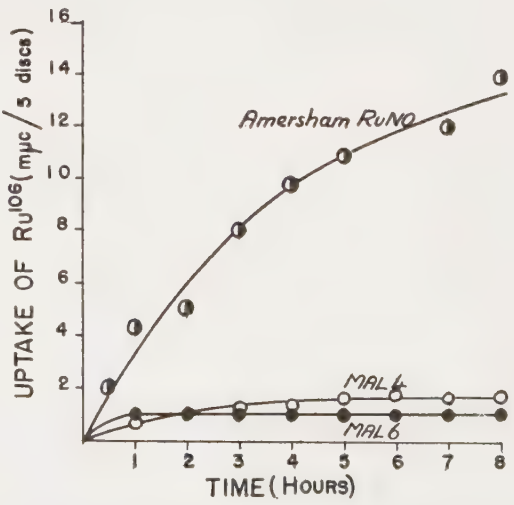

FIG. 12. Uptake of nitrosyl ruthenium of various origins by *Porphyra laciniata*.

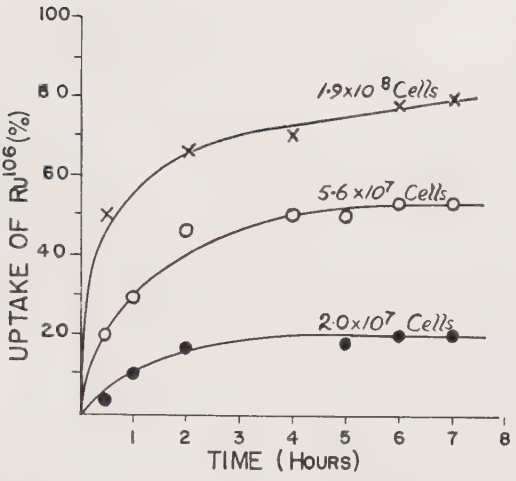

FIG. 13. Uptake of nitrosyl ruthenium by *Phaeodactylum tricornutum.*

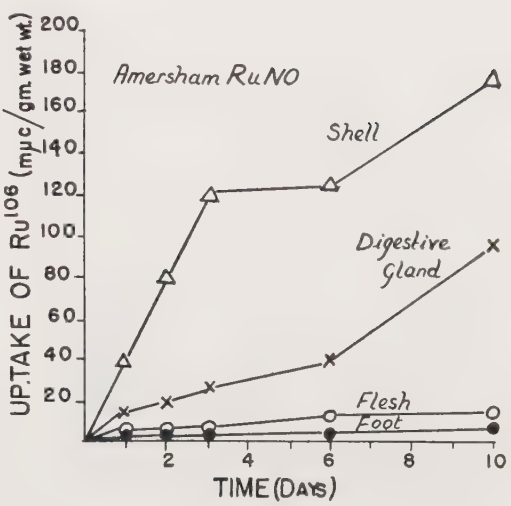

FIG. 14. Uptake of nitrosyl ruthenium by *Mytilus edulis.*

curred between living and dead cells, and, once adsorbed, the Ru^{106} was not readily removed from the cells by washing with sea water. The amount of Ru^{106} removed from the sea water solution increases with the number of diatom cells (Fig. 13).

Amersham nitrosyl Ru^{106} in sea water is accumulated by the marine mussel, *Mytilus edulis.* As can be seen from Figure 14, the shell of the animal accumulates a considerable proportion of the Ru^{106}. The digestive gland also accumulates the isotope. The foot and flesh are considerably lower in their accumulatory levels. Of the total nitrosyl Ru^{106} accumulated per mussel, as distinct from a specific weight basis, the shell accounts for some 95% of the total. Of the balance accumulated by the other organs of the animal the flesh accounts for some 60%, the digestive gland 35%, and the foot the remaining 5%.

When live mussels contaminated with nitrosyl Ru^{106} were placed in sea water free of Ru^{106}, the shell lost 50% of its Ru^{106} content within 24 hr, whereas the flesh lost less than 5%. In 7 days, however, the flesh had lost 20% and the shell 60%. Boiling the flesh in fresh water for 10 min resulted in a loss of 45% of its Ru^{106} content.

Neutralized MAL 6 of the same specific Ru^{106} content as the Amersham nitrosyl Ru^{106} is not accumulated by *Mytilus* to the same extent (Fig. 15). Although the shell is the major region of accumulation the amount is reduced by a factor of 5. The digestive gland, however, does not accumulate the Ru^{106} to any significant extent.

The uptake of Ru^{106} by various organs of plaice after immersion in sea water containing neutralized MAL 6 is shown in Table 3. Despite sampling errors of up to 50%, and apart from the contamination of the gills, gut and skin, there is some evidence of a concentration in the liver and

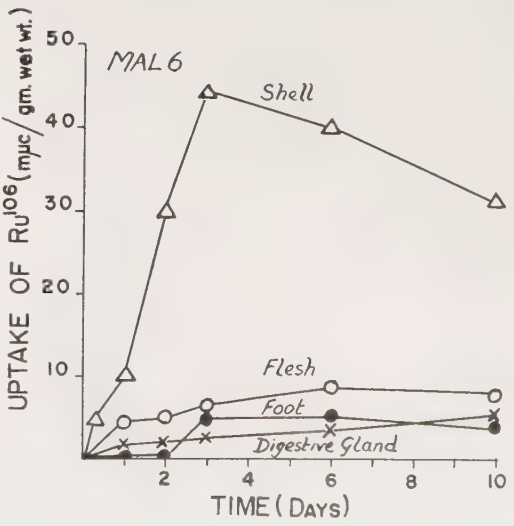

FIG. 15. Uptake of MAL 6 by *Mytilus edulis.*

TABLE 3. *Uptake of Ru^{106} by various organs of* P. platessa *after immersion in sea water containing neutralized MAL 6 (μc/g wet wt.)*

Organ	Background	1 day	4 days	7 days	14 days
Skin	2.6×10^{-6}	8.3×10^{-4}	2.1×10^{-4}	1.6×10^{-5}	1.7×10^{-3}
Flesh	4.0×10^{-5}	2.0×10^{-4}	1.9×10^{-4}	4.6×10^{-5}	3.5×10^{-4}
Gills	5.0×10^{-6}	4.2×10^{-3}	1.1×10^{-3}	7.4×10^{-4}	1.8×10^{-2}
Gut	3.3×10^{-6}	2.1×10^{-3}	5.4×10^{-4}	8.5×10^{-4}	6.5×10^{-3}
Liver	3.9×10^{-6}	1.4×10^{-4}	6.8×10^{-4}	1.1×10^{-3}	3.5×10^{-3}
Spleen	1.9×10^{-5}	7.8×10^{-5}	2.8×10^{-4}	4.5×10^{-4}	4.0×10^{-3}
Kidney	8.5×10^{-5}	1.2×10^{-4}	1.2×10^{-4}	6.4×10^{-4}	6.1×10^{-4}
Heart	1.2×10^{-4}	2.8×10^{-4}	2.8×10^{-4}	3.3×10^{-4}	1.3×10^{-3}
Gonads		1.1×10^{-4}	5.2×10^{-4}	2.4×10^{-4}	6.6×10^{-4}
Bone	2.3×10^{-5}	2.1×10^{-4}	4.7×10^{-5}	2.1×10^{-4}	5.1×10^{-4}

the spleen of the fish. Similar results have been obtained with Amersham nitrosyl Ru^{106} by D. H. Bratt of Research and Development, Windscale (private communication).

Analyses were made of the radioactive content of various organs of plaice caught in the vicinity of St. Bees Head to Whitehaven (9 miles north of Sellafield) and from near the pipeline end at Sellafield. The gut contents of the fish closely resembled the composition of the sea bed from the two stations. Those from the St. Bees Head–Whitehaven catch contained mainly *Ensis* sp. and a little sand, whereas the fish caught off Sellafield contained *Amphiura filiformis* and fine silt. Table 4 shows that although the level of activity for most of the organs and flesh of the fish from the two regions are similar, the intestine and gut contents of those fish caught near the pipeline end are about 10 times more radioactive. As to the bone values, it is doubtful if the difference was due to Ru^{106}, but it may well have been due to other fission products in the effluent. Because of the relatively low levels of radioactivity and small quantity of available material, individual isotopic analysis proved difficult.

Investigation of nitrosyl ruthenium species.

Because of the difference in behavior between the Amersham nitrosyl Ru^{106} in sea water and the Ru^{106} present in the MAL 4 and MAL 6 solutions in sea water, it was considered necessary to investigate the nature and form of the various ruthenium species present in these solutions. This was

TABLE 4. *Comparison of radioactivity in* P. platessa *caught off St. Bees Head and near the pipeline end at Sellafield (May 1957)*

Organ of fish	St. Bees Head		Sellafield	
	μc/g wet wt.	μc/fish	μc/g wet wt.	μc/fish
Intestine	7.6×10^{-6}	3.3×10^{-5}	4.1×10^{-5}	1.3×10^{-4}
Gut contents	3.1×10^{-5}	2.9×10^{-4}	1.3×10^{-4}	1.4×10^{-3}
Liver	7.4×10^{-6}	2.7×10^{-5}	1.5×10^{-5}	2.6×10^{-5}
Spleen	1.7×10^{-5}	3.0×10^{-6}	1.5×10^{-5}	1.1×10^{-6}
Heart	6.6×10^{-6}	7.9×10^{-7}	8.5×10^{-5}	9.4×10^{-6}
Gonads	5.7×10^{-6}	7.1×10^{-6}	1.0×10^{-5}	6.8×10^{-6}
Kidneys	7.4×10^{-6}	7.0×10^{-6}	9.2×10^{-6}	7.8×10^{-6}
Gills	7.3×10^{-6}	6.0×10^{-6}	8.2×10^{-6}	5.3×10^{-6}
Flesh	3.0×10^{-6}	4.9×10^{-4}	3.3×10^{-6}	5.6×10^{-4}
Skin	2.9×10^{-6}		4.6×10^{-6}	
Bone	1.9×10^{-5}	3.3×10^{-4}	5.1×10^{-6}	5.4×10^{-5}
Whole fish	—	1.2×10^{-3}	—	2.2×10^{-3}

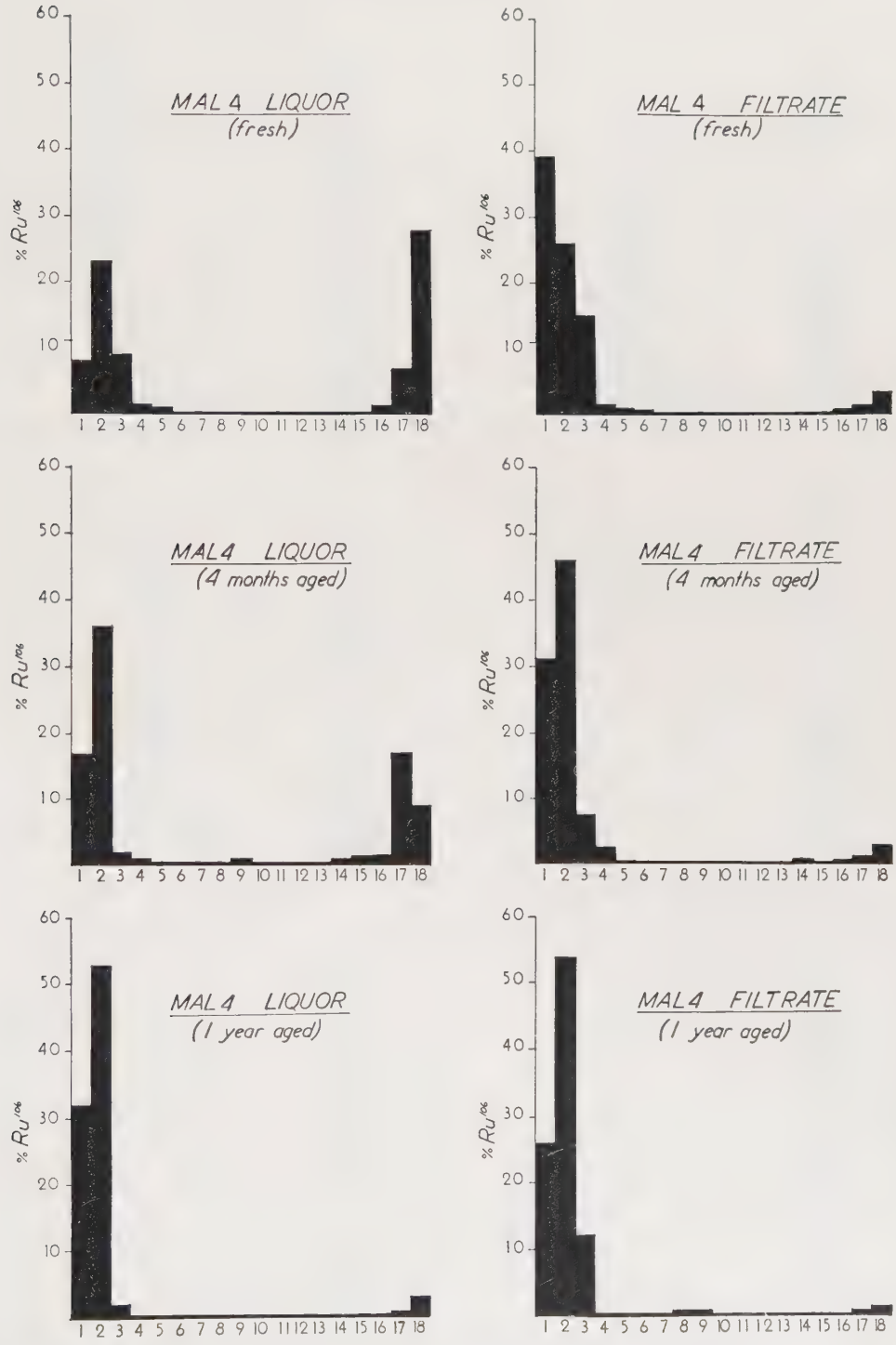

FIG. 16. Strip chromatograms showing distribution of nitrosyl Ru^{106} species in MAL 4 liquor and MAL 4 filtrate.

FIG. 17. Strip chromatograms showing distribution of nitrosyl Ru^{106} species in MAL 6 liquor and MAL 6 filtrate.

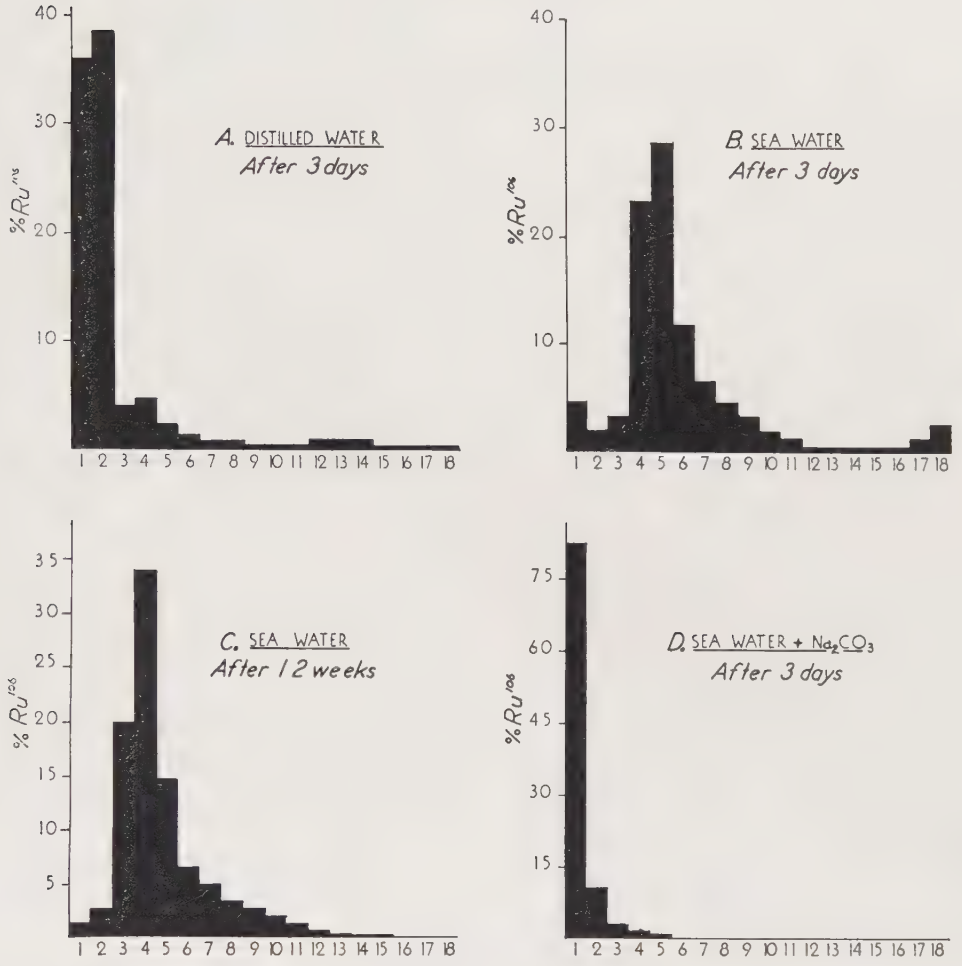

FIG. 18. Strip chromatograms showing distribution of Amersham nitrosyl Ru^{106} species in distilled water and sea water.

carried out by the chromatographic method outlined above.

Figure 16 clearly shows the presence of more than one species of nitrosyl ruthenium in the original MAL 4 raffinate both when fresh and after aging for 4 months. After storage for over a year, however, only one species of nitrosyl ruthenium (Rf = 0.1) is found. Only this same single species is found in the filtrates of the neutralized MAL 4 solution at all ages. Unlike MAL 4, MAL 6 (Fig. 17) is characterized by this single species of nitrosyl ruthenium (Rf = 0.1) both in the original raffinate after all storage periods, and in the respective filtrates of the neutralized raffinates. The behavior of the Amersham nitrosyl trinitrate solution was found to be quite different following dilution (100 ×) with distilled water from that following similar treatment with sea water. In distilled water, the trinitrato species was rapidly hydrolyzed to the non-nitrato form, which remained stable (Fig. 18A). In sea water, however, the hydrolysis of the trinitrato species was slower and although after 6 hr some non-nitrato species were present, as time elapsed a single species of Rf = 0.2 was formed. This species became dominant after 24 hr, and after 3 days quite stable, accounting for some 70% of the total radioactivity (Fig. 18B). Even after 12 weeks the same single species was

present, again accounting for 75 to 80% of the total radioactivity (Fig. 18C). When the Amersham nitrosyl trinitrato solution was diluted (100 ×) with sea water and then brought to pH 8.0 with Na_2CO_3, a rapid hydrolysis to the non-nitrato or polynuclear species occurred (Fig. 18D).

Dr. P. G. M. Brown of the A. E. R. E., Harwell, was kind enough to synthesize ruthenium nitrosyl chloride. After standing for 3 days in 3% NaCl and then being chromatographed with methyl isopropyl ketone (equilibrated with 3M HNO_3), a solution of this chloride gave an Rf value of 0.2 thereby confirming the species described above as ruthenium nitrosyl chloride.

SUMMARY

1. The uptake of Amersham nitrosyl Ru^{106} by fine particles of sand and silt is an adsorption process.

2. The Amersham nitrosyl Ru^{106} exhibits greater adsorption on to sand particles than the neutralized solutions MAL 4 and MAL 6 of the same specific activity.

3. Nitrosyl Ru^{106} forms an insoluble complex with ferric hydroxide. The presence of a surface layer of ferric hydroxide on sand and silt particles greatly enhances the quantity of nitrosyl Ru^{106} adsorbed. Once adsorbed, the Ru^{106} appears to form a complex with the ferric layer and is difficult to remove.

4. The uptake of nitrosyl Ru^{106} by *Porphyra laciniata, Ulva lactuca,* and *Laminaria digitata* is also a surface phenomenon. The Ru^{106} complexes with the extra-cellular polysaccharide material, and differences in accumulation of the nuclide may be due to differences in the chemical nature of this extra-cellular material. The diatom, *Phaeodactylum tricornutum,* also accumulates nitrosyl Ru^{106} by adsorption. As with sand particles, the Amersham nitrosyl Ru^{106} is accumulated by marine algae to a higher degree than is MAL 4 or MAL 6 of the same specific activity.

5. Amersham nitrosyl Ru^{106} and neutralized MAL 6 are accumulated by *Mytilus edulis.* Adsorption on to the shell of the animal accounts for the major fraction of accumulated radioactivity.

6. Accumulation of fission products by a bottom-feeding marine fish, *Pleuronectes platessa,* appears to be related to the nature of the sea bottom material and the invertebrates living within this material. Plaice (*P. platessa*), feeding on living organisms embedded in fine silt, take in the particles, which by nature of their large surface area are relatively highly contaminated with fission products. Contamination of gills, gut and skin occurs when plaice are kept in sea water containing neutralized MAL 6 and Amersham nitrosyl ruthenium. There is some evidence of a concentration in the liver and spleen of the fish. Very little, if any, Ru^{106} is detected in the muscle of the fish.

7. Chromatographic analyses reveal differences in the species of nitrosyl Ru^{106} found in MAL 4, MAL 6 and Amersham trinitrato nitrosyl ruthenium. On the addition of the Amersham form to sea water ruthenium nitrosyl chloride is formed. The differences in uptake from sea water, by the various organisms and particles described, of Amersham nitrosyl Ru^{106} and the Ru^{106} from MAL 4 and MAL 6, may therefore be explained on the basis that different physical and chemical forms of ruthenium are present.

8. The present results indicate that, in laboratory studies on the fate of radioactive effluents discharged to aquatic environments, one should use either factory plant solutions following effluent treatment or pure solutions of the particular nuclide altered to conform to the physical and chemical nature of the nuclide present in the effluent.

REFERENCES

Dunster, H. J. and F. R. Farmer. 1958. A summary of the biological investigations of the discharges of aqueous radioactive waste to the sea from Windscale Works, Sellafield, Cumberland. U.K.A.E.A. Industrial Group Report (IGS-R/R-3).

Eppley, R. W. and L. R. Blinks. 1957. Cell space and apparent free space in the red alga *Porphyra perforata.* Plant. Physiol., **32**: 63–64.

FLETCHER, J. M., I. L. JENKINS, F. M. LEVER, F. S. MARTIN, A. R. DOWELL, AND R. TODD. 1955. Nitrato and nitro complexes of nitrosyl ruthenium. J. Inorganic and Nucl. Chem., **1**: 378–401.

KETCHUM, B. H. AND A. C. REDFIELD. 1938. A method for maintaining a continuous supply of marine diatoms. Biol. Bull., **75**: 165–169.

SADDINGTON, K. AND W. L. TEMPLETON. 1958. Disposal of Radioactive Waste. George Newnes Ltd., London. 102 pp.

WAIN, A. G., P. G. M. BROWN AND J. M. FLETCHER. 1957. The chromatographic separation of nitrate complexes of nitrosyl ruthenium in nitric acid solutions. Chem. and Ind., pp. 18–20.

BIOTIC CHANGES IN A BAY ASSOCIATED WITH THE END OF A DROUGHT[1]

H. Dickson Hoese

Virginia Fisheries Laboratory

ABSTRACT

Heavy rainfall in early 1957 broke the most severe drought in the history of Texas. Resultant heavy river discharge reduced salinities in Mesquite Bay on the Central Texas Coast by over 30‰ in two months. Effects on the bay fauna were catastrophic with complete mortality of stenohaline marine sessile and infaunal mollusks. The flora and fauna of the high salinity drought period and of the subsequent low salinity period are compared. A high salinity *Ostrea equestris-Brachidontes exustus* community was replaced in 1957 by a *Crassostrea virginica-Brachidontes recurvus* community. The rapid lowering of salinity apparently did not kill fishes and other motile forms; in most cases these must have escaped by moving out of the area, but several stenohaline species were found in very low salinities immediately after the influx of fresh water. Mortality of euryhaline mollusks occurred in 1958 when salinities fell below 3‰. Some relations of organisms to salinity are discussed from ecological and paleoecological viewpoints.

INTRODUCTION

From 1948 (and especially from 1950) through 1956, the worst drought recorded in the history of Texas had many effects, not only on land, but in the shallow coastal bays as well. It was noted by many authors that salinities in the Central Texas bay system were rising during the drought (Parker 1955). These bays are primarily under the influence of the Guadalupe River system (Collier and Hedgpeth 1950; Parker 1955; Simmons and Hoese 1959). Low rainfall over the Guadalupe drainage and the resultant reduced river discharge, plus increased evaporation from higher temperatures, explain the high salinities which finally reached levels above oceanic salinity.

During the drought, marine stenohaline forms invaded the bays (Parker 1955 and unpublished), apparently because of the removal of this low salinity barrier. During the less extreme portion of the drought the benthic invertebrate fauna was studied by Puffer and Emerson (1953), Parker (1955) and others. There has been no report on conditions during the extreme part of the drought in 1956 nor on conditions in 1957 and 1958 when salinities were much lower than in previous years.

This paper records the effects of the rapid lowering of salinity in 1957 on the flora and fauna of Mesquite Bay, Texas, and compares the drought-induced high-salinity flora and fauna with that present during the low salinities after the drought ended.

Data are from a Master of Science thesis submitted to the A. & M. College of Texas, which was also submitted as a report of the Mesquite Bay Project to the Marine Fisheries Division of the Texas Game and Fish Commission. The work was done under the direction of Dr. Sewell H. Hopkins, whose advice and criticisms are gratefully acknowledged. Mr. Howard T. Lee and the Texas Game and Fish Commission Marine Laboratory staff under his direction assisted greatly in many ways. Mr. Robert H. Parker of Scripps Institution of Oceanography kindly contributed information on the benthic fauna of Mesquite Bay during the drought. Mr. Richard B. Moore assisted the study during the summer of 1957, and was responsible for many analyses of dead mollusks. I am indebted to the following people for identifications: Dr. John T. Conover, algae; Dr. Willard D. Hartman, sponge; and Mr. Charles B. Goodwin, crustaceans. Mr. Roy W. Spears deter-

[1] Contribution No. 48 from the Marine Laboratory, Texas Game and Fish Commission, Rockport, Texas.

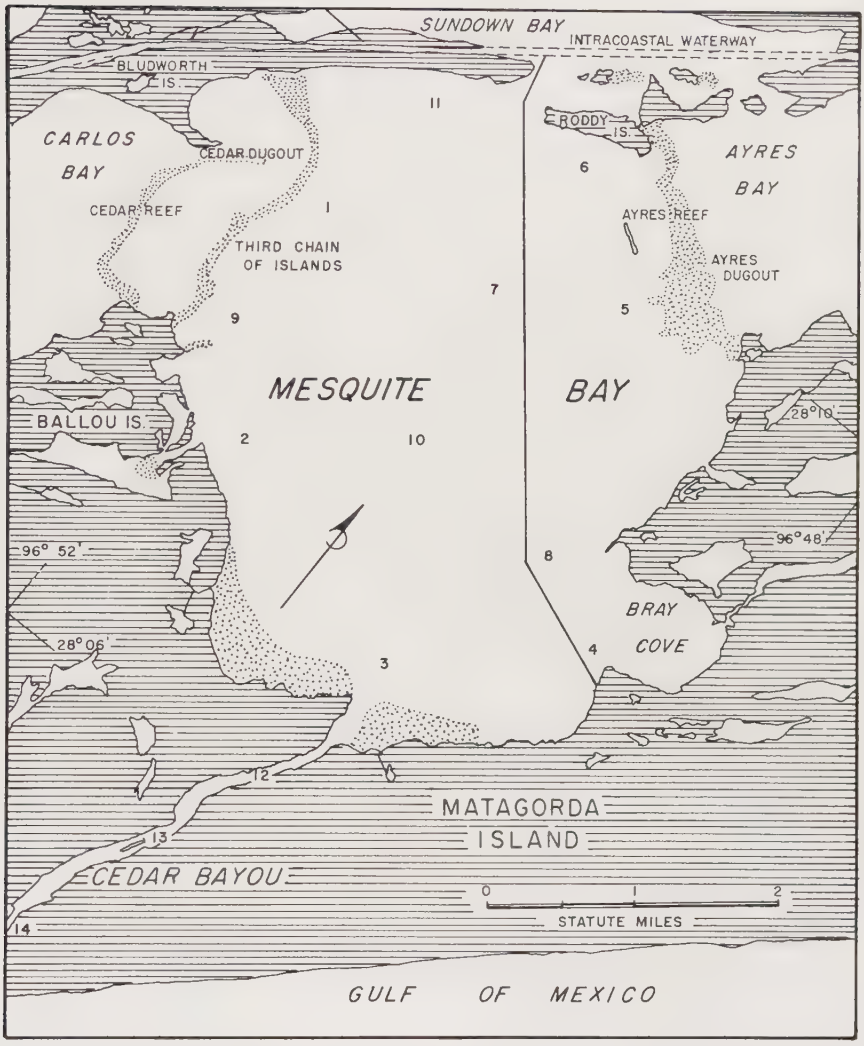

FIG. 1. Mesquite Bay and Cedar Bayou showing features of study area and stations occupied. Arrow points to true north. Heavy line is a partly shoaled channel, marked by piling.

mined salinities, pH and turbidity. Mr. James R. Dixon assisted in many collections during the earlier parts of the study.

METHODS

During the main study (June, 1957–May, 1958) one collection was made each month with a ten-foot otter trawl at each station in Mesquite Bay and Cedar Bayou (Fig. 1). The trawl had 1.75 inch stretched mesh and was pulled in a circle by an inboard cruiser for 10 min at each station. Other trawls, gill nets, trammel nets, seines, dip nets, and hook and line were used to sample species not normally taken in regular trawl collections. Mollusks were sampled with Ekman and oyster dredges.

Four days were spent collecting during the drought (November 9, 1956; January 20, February 11, and March 22, 1957). Salinities were determined by the Mohr method of silver nitrate titration. They are presented in parts salt per thousand parts sea water. Thermometer temperatures were read to 0.1°C. A Beckman model G

pH meter was used to measure hydrogen ion concentration. Turbidities were measured from surface water samples with a Lumitron Colorimeter, using distilled water as a standard and are expressed as percentage light transmission. Final identifications of all the fishes and many invertebrates were made by the author. Most specimens were identified in the field, but representatives of many species were deposited in the Museum of the Marine Laboratory, Rockport. For more details of the study the original thesis may be consulted.

AREA STUDIED

A detailed description of the area has been given by Simmons and Hoese (1959). Mesquite Bay, located at about 96° 50′ W, 28° 10′ N (Fig. 1), receives its drainage from what Thornthwaite (1948) considered to be the dry subhumid belt before the drought. The center of the bay is 24.8 miles from Aransas Pass (inlet) and 30.6 miles from Pass Cavallo. Cedar Bayou, a narrow three-mile tidal inlet originally connected the bay with the Gulf of Mexico but has recently shoaled, and during most of this study there was no direct water exchange with the Gulf of Mexico. As a result there were no astronomical tides in the bay.

Mesquite Bay is a small, oval, partly occluded bay separated from Aransas and San Antonio bays by two smaller bays and by chains of oyster reefs. The long axis of the bay is perpendicular to the Intracoastal Waterway in this area and is 4.6 miles in length. The greatest width is 3.8 miles. Collier and Hedgpeth (1950) give the area as 13.5 square miles.

The basin is shallow with a saucer-shaped profile. U. S. Coast and Geodetic Survey Maps list the maximum depths as 4.5 ft at mean low tide. Water levels are raised as much as 2 ft during periods of heavy runoff and high tides. Cedar Bayou is deeper over most of its length with places as deep as 9 ft.

The recent sediments of Mesquite Bay are largely clayey sands, except for a band of sand from the vicinity of Station 2 alongshore through Station 3 to near 5 (Shepard and Moore 1955). The bottom of Cedar Bayou was mostly reduced unconsolidated mud with large amounts of sulfides and sulfate-reducing bacteria.

SALINITY

Salinity data for Mesquite Bay in the past have been too meager to permit an exact statement of what is normal. Until the drought the bay had low to medium salinities (Galtsoff 1931; Parker 1955). Parker (1955) summarizing previous data, stated that salinities in Mesquite Bay during 1926, 1927, 1936, and 1937 ranged between 4 and 19‰ and during 1947–50 from 14 to 28‰. During his survey (1950–53) salinities never fell below 22‰ and once exceeded 40‰. In 1956 the drought reached an extreme, which was reflected in Mesquite Bay salinities from August, 1956 through March, 1957. During this period 28 salinities were determined. One hydrometer reading taken in August, 1956 showed a salinity slightly over 50‰. At this time Aransas Bay salinities ranged up to 45‰ (Hoese and Moore 1958). On November 9, 1956, salinities taken at three stations ranged from 44.5 to 45.3‰. In 1957, eight salinities averaged 37.2‰ on January 29, five averaged 39.3‰ on February 11, and four averaged 35.6‰ on March 22. Such high salinities have been previously found only in the Laguna Madre and Baffin Bay area on the Texas Coast (Simmons 1957; Breuer 1957).

Data are again available for May 13, 1957, when salinities were dropping rapidly, particularly in the upper bay. The salinity ranged from 5.1‰ at Station 6 to 12.2 ‰ at Station 3. By May 29 readings had fallen to 2.3 to 2.9‰ throughout the whole bay. The salinity then rose gradually to an average of 5.4‰ for June, but fell to a low for the year of 1.7‰ on June 27. Then salinities suddenly rose to 12‰ because of an influx of Gulf water caused by Hurricane Audrey.

Through August salinity rose rapidly, reaching a high of 33.4‰ on August 15, then steadily decreased to near 7‰ in early January. Then a mass of saltier water was

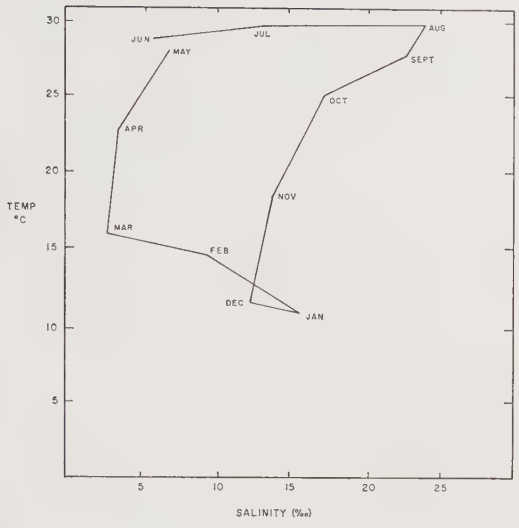

FIG. 2. Average temperature-salinity diagram for Mesquite Bay, June, 1957 through May, 1958.

blown into the bay causing a temporary rise. After this, salinity again dropped rapidly and remained below 5‰ during March and April. The lowest salinities ever recorded for the area were taken on March 14, 1958. These were 0.5‰ at Station 6 and 0.9‰ at Station 11. Salinity rose slightly in May but remained between 5 and 10‰ until the study was terminated on June 2, 1958. An average temperature-salinity diagram for June, 1957–May, 1958 is given in Figure 2. Additional data are presented by Simmons and Hoese (1959).

THE HIGH SALINITY OR DROUGHT FAUNA AND FLORA

Even though the four days spent collecting during the drought were not adequate to determine all species present, the major organisms of the bay were discovered. More detailed descriptions of the drought fauna had been published earlier by Puffer and Emerson (1953) and Parker (1955).

Algae were not usually identified to species in the field. Algae were observed in the clear waters of the bay during the drought. They formed large, clumped masses over most of the area. Most conspicuous in trawl collections were *Gracilaria blodgetti* Harvey, *G. folliifera* (Forsskal) Boergesen, and *G. verrucosa* (Hudson) Papenfuss. Other species formed a smaller mass but were repeatedly taken: *Hypnea musciformis* (Wulfen) Lamouroux, *Dictyota dichotoma* (Hudson) Lamouroux, *Agardhiella tenera* (J. Agardh) Schmitz, and *Ulva lactuca* Linn.

One spermatophyte, *Diplantheria wrightii* (Ascherson) Ascherson, was growing abundantly along the submerged shore of Cedar Bayou and the southern shore of the bay.

Parker (1955) had noted many faunal changes in the Central Texas Bay System which were associated with rising salinities earlier in the drought. Essentially, much of the polyhaline fauna found in lower Aransas Bay in 1940–41 by Ladd (1951) had spread over the whole area. At least three low salinity forms, *Rangia cuneata* Gray, *Littordina sphincostoma* Abbott and Ladd, and *Brachidontes recurvus* Rafinesque, apparently suffered complete mortality over much of their original range. Populations of other forms were reduced sharply. The fauna of Mesquite Bay in 1956 and early 1957 was predominantly marine, but a few euryhaline types remained in some numbers.

The infauna of Mesquite Bay in 1956–57 was composed largely of a *Mercenaria campechiensis-Chione cancellata* community, with scattered populations of *Tagelus divisus* Spengler and *Trachycardium muricatum* Linnaeus. At least two estuarine species survived in high salinites, *Ensis minor* Dall and *Mulinia lateralis* Say. *M. lateralis* is found in the Upper Laguna Madre (Simmons 1957), and according to Pulley (1952) *E. minor* lives in all the bays in high salinity. Hinged pairs of valves of other mollusks which had apparently lived in the bay during the drought were taken after the drought ended. These are listed in Table 2.

Reefs of dead *Crassostrea virginica* (Gmelin) shells formed the base for an *Ostrea equestris-Brachidontes exustus* community, and there was later evidence that *Crepidula plana* Say was abundant on the reefs. Live populations of *C. virginica* were found only on Ayres Reef where many large, old individuals persisted.

TABLE 1. *Comparison of flora found in Mesquite Bay during and after the drought, with salinity ranges*

Drought (Nov., 1956–March, 1957)	Low Salinity (Sept., 1957–May, 1958)	Both
Ulva lactuca (36.0–38.3‰)	*Enteromorpha clathrata* (0.5–17.0‰)	*Cladophora fascicularis*
Dictyota dichotoma (34.6–40.6‰)	*Ulva fasciata* (3.0–17.0‰)	*Gracilaria foliifera*
Hypnea musciformis (35.7–40.1‰)	*Gelidium corneum* (1.8–3.0‰)	*Gracilaria verrucosa*
Agardhiella tenera (34.6–37.6‰)	*Gelidium crinale* (1.8–3.0‰)	
Gracilaria blodgetti (34.4–45.3‰)	*Gracilaria ferox*	
Laurencia poitei (36.0‰)	*Polysiphonia ferulaceae* (3.0–18.2‰)	
Polysiphonia denudata (40.1‰)	*Ruppia maritima* (0.5–18.3‰)	
Diplantheria wrightii (25.6–45.3‰)		

The bryozoan, *Zoobotryon pellucidum* Ehrenberg, was very abundant in November, 1956, and was taken in lesser quantities during the remaining months of high salinity. During the summer of 1958 great quantities drifted into the bays through Aransas Pass, but apparently salinities then were too low for it to reach the large population which it formed during the drought. Gunter (1950) reported small amounts of *Z. pellucidum* in Aransas Bay, and stated that it was abundant only in the Gulf.

A sponge, with three inquilines, *Petrolisthes armatus* (Gibbes), *Alpheus armillatus* (Milne Edwards), and *Neopanope texana* (Stimpson), formed large colonies in the bay, particularly at Stations 4 and 5. Dr. Hartman (personal communication) has identified the sponge as *Suberites undulatus* George and Wilson, which he has also identified from Matagorda Bay. The sponge was first found by George and Wilson (1919) in pools at low tide, and was described as gray. It is interesting to note that the Mesquite Bay sponge was orange, but it turned gray when it was dying in the low salinities of June, 1957.

Motile invertebrates and fishes were not adequately sampled during the drought. *Penaeus aztecus* Ives, *P. setiferus* (Linnaeus), *P. duorarum* Burkenroad, *Callinectes danaë* Smith, *Micropogon undulatus* (Linnaeus), *Leiostomus xanthurus* Lacépède, and *Lagodon rhomboides* (Linnaeus) were taken most often. One stenohaline fish was taken only during the drought, but it is probable that adequate sampling would have revealed many stenohaline marine fishes in the area. Young *Chloroscombrus chrysurus* Linnaeus were abundant in November, and it was reported that large pompano, probably *Trachinotus carolinus* (Linnaeus), were taken by fishermen from the reefs in nearby San Antonio Bay. Gunter (1945) did not take *C. chrysurus* in salinities below 16.5‰ and did not find *T. carolinus* below 28.1‰.

MASS MORTALITY IN THE 1957 PERIOD OF LOW SALINITY

Between March 22 and May 29, 1957, salinities in Mesquite Bay dropped from over 35‰ to less than 3‰. This was caused by flooding of the Guadalupe River system, which empties into San Antonio Bay, less than 10 miles from Mesquite Bay. The average discharge for the Guadalupe River system during the months of March, April, and May was over 8,000 cfs, more than ten times what it had been in 1956.

The marine infaunal community suffered complete mortality. Attached valves of *Mercenaria campechiensis, Chione cancellata, Tagelus divisus,* and *Trachycardium muricatum* were taken in great numbers in trawls. A few small *M. campechiensis* were taken alive, and some dead ones still had pieces of meat attached to the valves; apparently they had migrated to the surface of the bottom when salinity dropped and had finally died. There was evidence of crab predation on several valves of *M. campechiensis* and *C. cancellata,* probably due to *Callinectes sapidus* Rathbun, which was the only large crab found in the bay at the time. It has previously been reported that *C. sapidus* preys on hard clams (Haven and Andrews 1957).

Since only the smaller *M. campechiensis* were taken alive in late May and early June,

TABLE 2. *Comparison of invertebrates taken during and after the drought, with lowest and highest salinity in which living individuals were found*

Drought (Nov., 1956–March, 1957)	Low Salinity (May, 1957–May, 1958)
Suberites undulatus (34.6–44.5‰)	*Cliona truitti* (16.0‰)
Cliona celata (a)	*Dactylometra quinquecirrha* (13–26‰)
Aurelia aurita (21.2–44.5‰) (c)	*Stylochus* sp. (3.0‰)
Stomolophus meleagris (44.5‰)	*Erichsonella attenuata* (2.4–13.4‰)
Beroë ovata (37.5–45.3‰) (d)	*Alpheus heterochaelis* (3.0–16.0‰)
Alpheus armillatus (34.6–44.5‰)	*Macrobrachium ohione* (3.7–5.0‰)
Petrolisthes armatus (5.3–35.7‰) (b,c)	*Rithropanopeus harrisi* (2.0–7.0‰)
Neopanope texana (3.0–45.3‰) (b,c)	*Molgula* sp. (6.4–15.6‰)
Menippe mercenaria (14.0–38.8‰) (c)	*Brachidontes recurvus* (0.5–27.0‰)
Libinia dubia (20.4–38.8‰) (c)	*Crassostrea virginica* (0.5–27.0‰)
Squilla empusa (15.6–34.6‰) (c)	*Pagurus floridanus* (13.2–23.8‰)
Zoobotryon pellucidum (35–45.3‰)	
Dentalium texasianum (a)	Both
Crepidula plana (12.0–45.3‰) (b,c)	
Anachis obesa (35.8–39‰)	*Mnemiopsis mccraydi* (4.9–40.1‰)
Brachidontes exustus (12–45.3‰) (b,c)	*Penaeus setiferus* (2.9–45.3‰)
Ostrea equestris (34.6–45.3‰)	*Penaeus aztecus* (0.5–45.3‰)
Trachycardium muricatum (a)	*Penaeus duorarum* (2.7–35.7‰)
Chione cancellata (38.5‰) (a)	*Clibinarius vittatus* (0.5–45.3‰)
Dosinia discus (a)	*Callinectes sapidus* (2.8–40.6‰)
Mercenaria campechiensis (34.4‰) (a)	*Callinectes danaë* (4.7–45.3‰)
Tagelus divisus (35.6‰) (a)	*Mulinia lateralis*
Ischnochiton papillosa (38.5‰)	*Ensis minor*

a = Represented by recently killed specimens after influx of fresh water in June, 1957.
b = Few taken in low salinities in June, 1957 immediately after salinities had fallen. Not taken later in comparable low salinities.
c = Abundant during drought, returning during medium to high salinities in later summer and fall of 1957. Absent from low salinities in spring of 1958.
d = One specimen taken in a salinity of 17.3‰.

there must have been a size differential in death rates due to lowered salinity. The attached valves fell into three length groups with modes near 40, 80, and 120 mm. Several smaller live clams were transported to the laboratory and placed in sea water, but they did not survive.

Complete mortality of *Ostrea equestris* had occurred on the reefs by June, 1957 and extensive mortality of *Brachidontes exustus* was observed. A few *B. exustus* survived until March of 1958 when salinities again became extremely low.

At the end of June, 1957, a washover from the high tides of Hurricane Audrey raised salinities from 2 to 12‰. Salinities in Mesquite Bay rose rapidly during the remainder of the summer to over 30‰ by late August. During this time a few marine fishes and marine mollusks reinvaded the area. The mollusks were mostly reef forms, which attached to the oyster shells of the reefs, and to the new beds of *Mercenaria* shells which was formed by the extensive spring mortality of *M. campechiensis*. The new marine invasion was not large, but included *Crepidula plana, Squilla empusa, Pagurus floridanus, Menippe mercenaria, Callinectes danaë, Petrolisthes armatus, Neopanope texana,* and serpulids. The rise in salinity apparently prevented further mortality of *Brachidontes exustus,* and allowed reproduction, for these mussels were eventually found on the new clam-shell beds as well as on the old oyster beds.

MASS MORTALITY IN THE 1958 PERIOD OF LOW SALINITY

Salinities dropped in late February to below 10‰, and in March to below 5‰, where they remained until the middle of May, 1958. In the middle of March the whole bay had salinites less than 3‰ and salinities of 0.5 and 0.9‰ were recorded for stations 6 and 11 on March 14. In March the mor-

TABLE 3. *Salinity ranges of fishes taken more than five times in Mesquite Bay (Nov., 1956–May, 1958)*

Carcharinus leucas 7.5–17.6
Dasyatis sabina 2.6–45.3
Dorosoma petenense 7.6–16.8
Synodus foetens 14.3–44
Bagre marina 4.9–26.5
Galeichthys felis 2.8–44.6
Urophycis floridanus 3.0–12.3
Syngnathus scovelli 3.0–45.4
Fundulus similis 5.0–34.4
Fundulus grandis 5.0–34.4
Cyprinodon variegatus 5.0–34.4
Mugil cephalus 4.0–40.6
Polydactylus octonemus 4.5–7.0
Orthopristis chrysopterus 3.0–44.5
Bairdiella chrysura 3.0–45.3
Stellifer lanceolatus 2.6–36.8
Leiostomus xanthurus 2.8–45.3
Cynoscion nebulosus 1.5–45.3
Lagodon rhomboides 2.5–45.3
Archosargus probatocephalus 8.6–36.8
Gobionellus hastatus 5.6–24.6
Prionotus tribulus 3.4–20.4
Citharichthys spilopterus 3.0–44.6
Etropus crossotus 2.6–30.0
Paralichthys lethostigma 2.6–30.0
Achirus lineatus 7.9–23.5
Symphurus plagiusa 3.3–44.5
Gobiesox strumosus 2.6–12.3
Opsanus beta 3.0–37.0
Porichthys porossissimus 4.4–44.5
Sphoeroides nephelus 5.5–45.3
Micropogon undulatus 1.5–45.3
Menticirrhus americanus 18.6–25.5
Cynoscion arenarius 5.3–25.5

tality of *Brachidontes exustus* and *Crepidula plana* was total, and many recently killed attached valves were found until the end of the study.

During the low salinities in March further mortality of other mollusks occurred. On March 21 salinities were 1.8‰ at Station 11 and 1.5‰ at Station 6 and many dying *Mulinia lateralis* and *Ensis minor* were taken. Here again the mollusks had migrated to the surface of the bottom of the bay to die. At other stations in the bay where salinities were 2.2 to 3.5‰ live individuals were found. However, additional heavy mortality of these two species was found later in March and early in April as far south as Stations 2, 10, and 8 where salinities had not fallen below 2‰, but had remained below 3‰ for at least a week. Parker (1955) stated that *M. lateralis* would tolerate salinities from 5 to 45‰, but it now seems that it is tolerant of salinities below 5 and probably down to 2 or 3‰. No mortality of *Mulinia* was recorded in June of 1957 when salinities were similar to those of March, 1958, and the higher temperatures of June (27.9–30.0°C) as compared to those of March (9.1–17.6°C) may have been involved; many marine animals seem to tolerate lower salinities at higher temperatures (Panikkar 1951).

Mortality of oyster spat (*Crassostrea virginica*) which had set the previous fall occurred during these low salinities. However, older oysters did not appear to be affected. Neither living specimens nor fresh shells of *Mercenaria campechiensis, Chione cancellata, Tagelus divisus, Trachycardium muricatum,* or *Ostrea equestris* was found after the 1957 low salinity kill, presumably because none survived or reinvaded the bay.

THE LOW-SALINITY FAUNA AND FLORA

A great difference in the fauna and flora was observed in early 1957 after the drought. Collecting effort during the low salinity period from June, 1957, through May, 1958, was much more intense than it was during the drought, so failure to find certain species that had been collected during the drought seems to be significant. On the other hand, it is probable that some of the forms found only in low salinities may have occurred in high salinities also, but were not taken or seen then because of less sampling.

The spat of *C. virginica* were first recorded in early September, 1957, when they were roughly 15–25 mm in valve length. It is suspected that these spat set in July and August; late May and June salinities were below 5‰, and spat also set on the valves of *Mercenaria* which were not available until late May. The young oysters grew rapidly. Seventy five placed in trays in the bay reached an average length of over 65 mm by May, 1958. Another spatfall

TABLE 4. *Animals taken in June low salinities, with lowest salinity recorded in literature*

Species	No. taken	June salinity	Lowest salinity in remainder of study	Lowest literature salinity
Orthopristis chrysopterus	7	3.0	12.6	10.3 (Gunter 1945)
Porichthys porossissimus	1	4.4	11.1	10.3 (Gunter 1945)
Opsanus beta	2	3.0	23.5	11.6 (Gunter 1945)
Callinectes danaë	38	4.4	5.8	16.5 (Gunter 1950)
Petrolisthes armatus	Few	5.3	15.0	?
Neopanope texana	Many	3.0	12.4	14.66 (Ryan 1956)

occurred later in 1957, but many of these spat were killed in March, 1958. *Brachidontes recurvus* also appeared in September and populations remained throughout the study. Essentially, the low-salinity reef community was composed of *Crassostrea virginica* and *B. recurvus,* but during the medium salinities from July, 1957, through February, 1958, marine forms such as *C. plana, B. exustus,* and serpulids existed in this community. The same community was also established on the new shell bed, formed by the recently killed valves of *Mercenaria campechiensis,* that now existed over most of the bay. During the low salinities of March-May, 1958, the marine component of the community was absent, with only *B. recurvus* and barnacles abundant on the reefs.

A sea squirt, probably *Molgula manhattensis* (DeKay), was a conspicuous member of the reef community from October through March. One large colony was also found on the plastron of a sacculinid-parasitized blue crab. The persistent *Mulinia lateralis* was abundant in low salinities, but most mollusks characteristic of the bay during the drought were absent.

The algae and higher plants were almost entirely different. *Ruppia maritima* Linnaeus replaced *Diplantheria. Gracilaria verrucosa* and *G. foliifera* were still abundant algae, but most species taken during the drought were absent, and were replaced by other forms (Table 1).

PALEOECOLOGICAL IMPLICATIONS

Certain of the data may have paleoecological bearing. Parker (1955) noted that many of the marine mollusks that invaded bays during the drought are found as fossils as far back as the Pliocene, and their presence in a fossil assemblage would be indicative of rising salinities. During the drought sedimentation was at a minimum, so there was little chance for burial of mollusks or other forms. The mass mortality was accompanied not only by lowered salinity but also by a large deposit of silt which eventually buried many mollusks. Gunter (1947) pointed out that many cases of mass mortality are accompanied by heavy rainfall and related increased chances for burial. But the picture is not simple. In Mesquite Bay, mortality occurred among euryhaline as well as marine forms, and these are presently mixed in the same sediment. Invasion of marine forms not only takes place over a long period of years, but occurs seasonally as well. During the high salinity summer of 1957, many marine forms invaded the bay and died at nearly the same time as two estuarine mollusks. However, only certain species reinvaded the bay. The *Mercenaria campechiensis-Chione cancellata* community should be recognizable in fossil assemblages; it would be indicative of high salinity conditions. Similarly the *C. virginica-Brachidontes recurvus* community and its high salinity counterpart, the *Ostrea equestris-B. exustus* community, may be keys to salinity conditions, with mixing of the species indicating intermediate conditions. Many valves of *Dinocardium robustum, Donax variablis,* and several *Arcas* were abundant on the Gulf beach near Cedar Bayou, but they were not found alive in Cedar Bayou or Mesquite Bay. Apparently, many species fail to enter bays for reasons other than the salinity difference.

Dredging to make a levee on Matagorda Island revealed many old mollusks of un-

certain age. One of the beds exposed contained many large, old *C. virginica, Cyrtopleura costata,* and *Dosinia discus.* All of these mollusks were present in Mesquite Bay during the recent drought. Such an assemblage might be indicative of high salinity bay conditions. Another nearby assemblage contained *D. robustum, D. variablis,* and *Arca* spp. which apparently did not enter the bays. This assemblage indicates the shallow Gulf of Mexico.

DISCUSSION

Several stenohaline marine invertebrates and fishes were taken in small numbers in June, 1957, immediately after the sudden drop in salinity (Table 4). However, they were conspicuously absent in similar salinities the following spring, a season when they are all known to inhabit bay waters in normal years. Apparently these animals had not had time to move out of the fresher water. Even though small numbers were taken, the few individuals present were of several species regarded by previous workers as not being euryhaline. None of the fishes was included by Gunter (1956) in his list of euryhaline species.

Unpublished experiments by the author and R. W. Spears in the summer of 1956 showed that several marine stenohaline fishes (*Orthopristis chrysopterus, Chilomycterus schoepfi, Chaetodipterus faber,* and *Synodus foetens*) would live in aquarium waters gradually diluted from 40–45‰ to 3–5‰, but when the salinity was reduced further, they would immediately die. To my knowledge, none of these species has been previously found in waters under 10 ‰. Simmons (1957) found that in the hypersaline Laguna Madre many species withstood rising salinities, but would not enter these same salinities from waters of lower salinity. It seems that the stenohaline species found in the low salinities of June, 1957, were within their tolerance limits, but were not within their preference limits. Other factors could have been involved; for instance, June temperatures were higher than those of the following spring, perhaps allowing easier adjustment to lower salinity. It seems probable also that the minimum salinity in which a stenohaline species is found will be lower when salinities are falling than when they are rising. The individuals found in low salinity were all apparently healthy juveniles. Gunter (1945 1950) found that the young of many species were found in the lowest salinities, and it was pointed out (Pearse and Gunter 1957) that this is also true of many stenohaline species.

During the spring of 1958, when salinities remained below 10‰, the fauna of the bay was largely composed of young individuals. Young *Callinectes sapidus, Micropogon undulatus, Leiostomus xanthurus,* and *Lagodon rhomboides* were abundant. The young of all these species except *L. xanthurus* were also found in March, 1957, in salinities of 34.6 to 35.7‰. The preponderance of young individuals in low salinities may not be due to salinity *per se,* but to some other correlated factor, such as a reduction in predation. Predators are important in the distribution of the oyster (Hopkins 1957). Reid and Hoese (1958) found that salinity was apparently not the major factor involved in size distribution of two species in East Bay, Texas, for the size distribution remained spatially the same in the bay after the salinity gradient had been considerably altered.

A list of species and the salinity range from which they were taken is shown in Tables 1, 2, and 3. Other than the stenohaline species listed in Table 4, one species was found in much lower salinities than previously recorded. Nine *Urophycis floridanus* (Bean and Dresel) from 80 to 195 mm were taken in February through May, 1958, in salinities of 3.0 to 12.3‰. Gunter (1945) did not take this species below 13.3‰.

Since most brackish-water animals are derived from marine stocks (Gunter 1945), the apparent upper limits of salinity tolerance indicated by the natural distribution of these forms in estuaries may normally be due to high-salinity predators and parasites, rather than real physiological limits. This seems to be the case with the oyster and it has been suggested for the hooked mussel

(Burkenroad 1931). On the other hand, the more marine forms such as *Ostrea equestris* and *Brachidontes exustus* seem to be excluded directly by low salinity, rather than by a secondary cause indirectly related to salinity.

The observed mass mortality of some species and the differences between the low and high salinity fauna and flora were presumably due mainly to salinity either directly or indirectly. However, other factors may have been important.

Turbidity is not only important to plants, but it seems to affect certain mollusks as well (Menzel 1955). During the present study higher turbidity coincided with lower salinity, except during the washover from Hurricane Audrey, and so their effects cannot be separated. During the high salinities, the waters were exceptionally clear, the bottom being visible over most of the bay. For the remainder of the study, surface turbidities ranged from 85% during periods of large river discharge to 99% during the small discharge of July and August, 1957. No turbidity values were obtained during the extreme high-salinity period but the water was usually almost as clear as the distilled water standard that was subsequently used. *C. virginica* is tolerant of higher turbidities than *O. equestris,* so mortality of the latter could theoretically have been caused by increased turbidity accompanying the lowered salinities.

The mass of *Gracilaria* spp. taken in trawls during high salinities was estimated to be at least 5 times that taken during low salinities. This could have resulted from turbidity restricting the penetration of sunlight and so production. Cystocarps of *Gracilaria* were found during both periods so apparently sexual reproduction was not affected.

Current patterns were presumably different, and with this the pattern of sedimentation. During the drought Mesquite Bay was essentially a negative estuary, losing more water by evaporation than it gained by river discharge and rainfall. During lower salinities in 1957 and 1958 the bay was flushing, except during part of the summer of 1957.

Values for pH were determined through a large range of salinities and varied from 7.0 to 8.7. The area is free from any man-induced pollution.

Two bibliographies on mass mortality have appeared recently (Geyer 1950; Brongersma-Sanders 1957). Severe cold, hypersalinity, and dinoflagellate blooms have been studied most as causes of mass mortality in Texas waters (Gunter 1952). They are more common, or perhaps more noticeable, there than kills due to fresh water. The mortality of 1957 may have been the worst ever caused by freshets in Texas bays, because of the severity of the drought and its rapid ending. No mass mortality of motile forms was noted, however, and it seems probable that they escaped to higher salinities.

REFERENCES

Breuer, J. P. 1957. An ecological survey of Baffin and Alazan Bays, Texas. Publ. Inst. Mar. Sci. Univ. Texas, **4**: 134–155.

Brongersma-Sanders, M. 1957. Mass mortality in the sea; *in* Treatise on Marine Ecology and Paleoecology. Geol. Soc. Amer., Memoir 67, **1**: 941–1010.

Burkenroad, M. D. 1931. Notes on the Louisiana Conch, *Thais haemostoma* Linn., in its relation to the oyster, *Ostrea virginica.* Ecology, **12**: 656–664.

Collier, A., and J. W. Hedgpeth. 1950. An introduction to the hydrography of tidal waters of Texas. Publ. Inst. Mar. Sci. Univ. Texas, **1**: 120–194.

Galtsoff, P. S. 1931. Survey of oyster bottoms in Texas. U. S. Bur. Fish. Invest. Rept., **6**: 1–30.

George, W. C., and H. V. Wilson. 1919. Sponges of Beaufort, N. C., Harbor and vicinity. Bull. U. S. Bur. Fish., **36**: 129–180.

Geyer, R. A. 1950. A bibliography on the Gulf of Mexico. Texas J. Sci., **2**: 44–93.

Gunter, G. 1945. Studies on marine fishes of Texas. Publ. Inst. Mar. Sci. Univ. Texas, **1**: 1–190.

———. 1947. Catastrophism in the sea and its paleontological significance, with special reference to the Gulf of Mexico. Amer. J. Sci., **245**: 669–676.

———. 1950. Seasonal population changes and distribution, as related to salinity, of certain invertebrates of the Texas Coast, including the commercial shrimp. Publ. Inst. Mar. Sci. Univ. Texas, **1**: 7–51.

———. 1952. The import of catastrophic mass mortalities for marine fisheries along the Texas Coast. J. Wildl. Manag., **16**: 63–69.

———. 1956. A revised list of euryhalin fishes of North and Middle America. Amer. Midl. Nat., **56:** 345–354.

Haven, D., and J. D. Andrews. 1957. Survival and growth of *Venus mercenaria, Venus campechiensis,* and their hybrids in suspended trays and on natural bottoms. Proc. Natl. Shell. Assoc., **47:** 43–49.

Hoese, H. D., and R. B. Moore. 1958. Notes on the life history of the bonnetnose shark, *Sphyrna tiburo.* Texas J. Sci., **10:** 69–72.

Hopkins, S. H. 1957. Interrelations of organisms. B. Parasitism: *in* Treatise on Marine Ecology and Paleoecology. Geol. Soc. Amer., Memoir 67, **1:** 413–428.

Ladd, H. S. 1951. Brackish-water and marine assemblages of the Texas coast with special reference to mollusks. Publ. Inst. Mar. Sci. Univ Texas, **2:** 129–162.

Menzel, R. W. 1955. Some phases of the biology of *Ostrea equestris* Say and a comparison with *Crassostrea virginica* (Gmelin). Publ. Inst. Mar. Sci. Univ. Texas, **4:** 68–153.

Panikkar, N. K. 1951. Physiological aspects of adaptation to estuarine conditions. Indo-Pacific Fisheries Council Proc., III, **1950:** 168–175.

Parker, R. H. 1955. Changes in the invertebrate fauna, apparently attributable to salinity changes in the bays of Central Texas. J. Paleont., **29:** 193–211.

Pearse, A. S., and G. Gunter. 1957. Salinity: *in* Treatise on Marine Ecology and Paleoecology. Geol. Soc. Amer., Memoir 67, **1:** 129–157.

Puffer, E. L., and W. K. Emerson. 1953. The molluscan community of the oyster reef biotope on the central Texas Coast. J. Paleont., **27:** 536–544.

Pulley, T. E. 1952. An illustrated checklist of the marine mollusks of Texas. Texas J. Sci., **4:** 167–199.

Reid, G. K., Jr., and H. D. Hoese. 1958. Size distribution of fishes in a Texas estuary. Copeia, **1958:** 225–231.

Ryan, E. P. 1956. Observations on the life histories of the Xanthidae (Mud Crabs) of Chesapeake Bay Amer. Midl. Nat., **56:** 138–162.

Shepard, F. P., and D. G. Moore. 1955. Central Texas coast sedimentation: characteristics of sedimentary environment, recent history, and diagenesis. Bull. Amer. Assoc. Petrol. Geol., **39:** 1463–1593.

Simmons, E. G. 1957. An ecological survey of the Upper Laguna Madre of Texas. Publ. Inst. Mar. Sci. Univ. Texas, **4:** 156–203.

———, and H. D. Hoese. 1959. Studies on the ecology and hydrography of Cedar Bayou, a natural tidal inlet on the Central Texas Coast. Publ. Inst. Mar. Sci. Univ. Texas, **6:** (In Press).

Thornthwaite, C. W. 1948. An approach toward a rational classification of climate. Geogr. Rev., **38:** 55–94.

NOTES AND COMMENT

On Winter Mixing and the Vertical Distribution of Phytoplankton[1]

During winter in the North Atlantic Ocean, the vertical distributions of temperature, salinity and other properties indicate that the surface waters are homogeneous, unstable and presumably well mixed to depths ranging from one to several hundred meters. Phytoplankton is seldom abundant at that time of year and under those conditions. Its paucity is usually attributed to the organisms being continually mixed through a column of water several times greater than that within which photosynthesis can occur (*i.e.* 25–75 m) and hence receiving insufficient light to grow. A number of authors have considered as a factor controlling plant growth, and particularly the onset of vernal flowering, the relationship between the depths of the euphotic and mixed layers. Vertical instability is identified with vertical turbulence, and hence the organisms are considered to be exposed to the average light intensity within the mixed layer, an intensity which must exceed some threshhold (*i.e.* compensation level) before growth may occur.

On March 27, 1957 an oceanographic station was occupied by R. V. Crawford at 39°27′N, 71°28′W, about 100 miles off New York City, in 1200 fathoms of water. Table 1 shows the vertical distribution to 200 m of temperature, salinity, density, dissolved oxygen, phosphate, nitrite, nitrate and total phosphorus. By all of these criteria the upper 100 m would be considered completely homogeneous. Such variations as exist in the data are irregular and inconsistent from one parameter to the next, and must therefore have resulted from sampling or analytical error. There are indications in the phosphorus analyses of a discontinuity at 150 m, and there is sharp break in the values of all properties at 200 m.

Examine now the vertical distribution of the phytoplankton within the upper 100 m of the same water column (Fig. 1). Only the five most abundant species (those with concentrations exceeding 1,000 cells/L at one or more depths) are shown. Not only is their vertical distribution uneven, but the pattern is different for each of the five species. We will not attempt to estimate how much of the observed variability in the phytoplankton may have resulted from counting error. Great as it may have been, no conceivable error could distort vertical homogeniety to the observed discontinuous distribution of the small, unidentified diatom, found only 10 m in a concentration of 12,300 cells/L.

Only one of the five species, *Coccolithus huxleyi*, is a motile organism. The other four species, all centric diatoms, had peaks of abundance respectively at 0, 10, 75 and 100 m. It seems doubtful that those found

[1] Contribution No. 1088 from the Woods Hole Oceanographic Institution.

Table 1. *The vertical distribution of physical and chemical properties in the upper 200 m at CRAWFORD Station 136 (39°27′N, 71°28′W) on March 23, 1957*

Depth (m)	Temperature (°C)	Salinity (‰)	Density (σt)	O_2 (ml/L)	PO_4-P µgA/L	NO_2-N µgA/L	NO_3-N µgA/L	Total P µgA/L
0	15.26	36.10	26.80	5.27	.36	.24	4.9	.50
10	15.24	36.10	26.79	5.18	.48	.28	6.0	.50
25	15.28	36.07	26.74	5.14	.48	.23	4.3	.51
50	15.23	36.07	26.76	5.09	.42	.26	4.2	.59
75	15.27	36.07	26.76	5.09	.48	.26	7.4	.53
100	15.23	36.05	26.76	5.14	.46	.26	6.0	.56
150	15.28	36.05	26.74	5.04	.68	.26	4.9	.69
200	13.33	35.71	26.90	3.97	1.02	.04	9.6	1.10

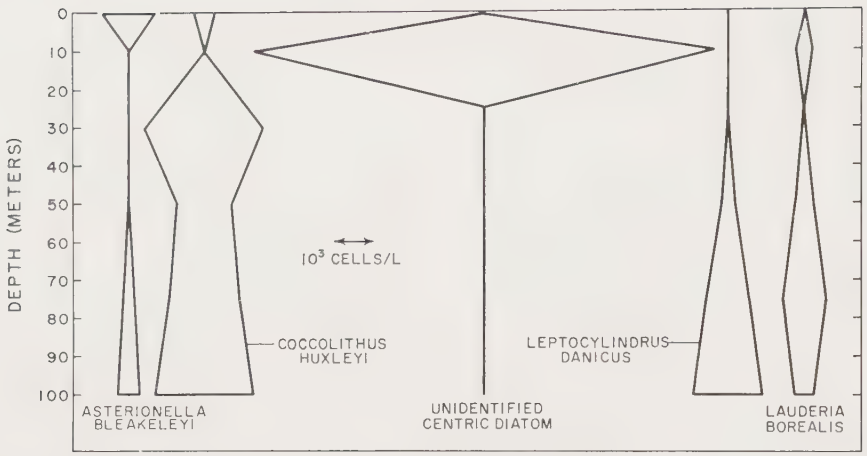

FIG 1. Vertical distribution of the five most abundant species of phytoplankton.

concentrated at 75 and 100 m grew at those depths. Indeed, the extinction coefficient ($k = .07$) indicates an euphotic zone of less than 75 m. Despite the appearance of a well mixed layer of over 100 m, vertical turbulence was apparently weak enough, for a long enough period, to permit not only the growth of populations of *Asterionella bleakeleyi* and the unidentified diatom in the upper 10 m, but also the growth and subsequent settling out of the euphotic zone of populations of *Leptocylindrus danicus* and *Lauderia borealis*. Thus a "mixed layer" by physical or chemical criteria is not necessarily mixed with respect to its phytoplankton, nor does it provide any indication of the occurrence or degree of mixing at the time of observation.

J. H. RYTHER AND E. M. HULBURT
Woods Hole Oceanographic Institution, Woods Hole, Mass.

WINTER OCCURRENCES OF *ANGUILLA ROSTRATA* ELVERS IN NEW ENGLAND AND MIDDLE ATLANTIC ESTUARIES[1]

The classic investigation of Johannes Schmidt (1925) traced the early life history of the American eel, *Anguilla rostrata*, from its spawning area centered north of the West Indies and southwest of Bermuda to New England coastal waters. Little, however, is known about the species following metamorphosis from the leptocephalus to the "glass eel" or elver stage which swims landward, ascends an estuary, and matures in a brackish or fresh-water habitat. Tremendous runs of elvers in New England and Middle Atlantic estuaries have been reported by various authors (Table 2). The enormity of the migration in certain areas has apparently led to the belief that the first elvers of the year arriving north of Chesapeake Bay are found in the spring (Bertin 1957, p. 169; Bigelow and Schroeder 1953, p. 152). The purpose of this note is to record winter occurrences and consider their significance. Contributions to the life history of the American eel are pertinent in view of Tucker's (1959) contention that "populations of the so-called 'European eels,' *A. anguilla*, are . . . entirely maintained by reinforcements of larvae of American '*A. rostrata*' parentage." The different interpretations need not concern us here, but serve

[1] Contribution No. 29 from the Narragansett Marine Laboratory. This investigation was supported by Office of Naval Research contract NR 083-054 and by Public Health Service grants RG-7003 and RG-5278.

TABLE 1. *Occurrences of* Anguilla rostrata *elvers in two Rhode Island estuaries and Raritan Bay, N. J.*

Date	Location	Length mm	Temperature °C
Jan. 3, 1955	Charlestown Pond, R. I.	49.0	7.0
Jan. 12, 1955	Pettaquamscutt River, R. I.	60.9, 56.0, 52.4	—
Feb. 25, 1958	Raritan Bay, N. J.	53.0	—
March 1, 1958	Raritan Bay, N. J.	58.5	2.0

TABLE 2. *Occurrences of* Anguilla rostrata *elvers along the Northeast Coast of North America*

Date	Location	Abundance	Author
Jan. 17–March 28, 1916–1922	Chesapeake Bay	—	Hildebrand and Schroeder (1928)
July–Aug.	Delaware Bay	thousands on tide flats	Fowler (1906)
April	New York area	common in estuaries	Nichols and Breder (1926)
Jan. 27, 1953	Long Island Sound	1 specimen	Wheatland (1956)
Feb. 25–April 16, 1958	Narragansett Bay	.002/m^3 average	Herman (1958)
Feb.–May, 1893–1907	Woods Hole	—	Fish (1925)
May 5 and 7, 1913	Little River, Mass.	"a tremendous run"	Bigelow and Schroeder (1953)
Summer	Streams in the Bay of Fundy region	—	Bigelow and Schroeder (1953)

to focus attention on the American representative of the genus *Anguilla*.

Occurrences of elvers taken during the course of plankton investigations in two Rhode Island estuaries and Raritan Bay, New Jersey are given in Table 1. The Rhode Island specimens were caught at night between 1945 and 2100 hr with a plankton net one-half meter in diameter that was suspended from a bridge and allowed to float in the tidal current. Dr. L. A. Stauber found the first Raritan Bay specimen stranded on a tide flat; the second was obtained with a Clarke-Bumpus plankton sampler.

The largest elver taken in the Pettaquamscutt River was examined prior to preservation and showed cranial pigmentation similar to the cerebral nerve cord spot characteristic of elver stage VB in Strubberg's (1913) classification of European eel developmental stages (see Bertin 1957; Fig. 44, p. 143). This specimen had 106 vertebrae. The other elvers became opaque before microscopic examinations could be made. Gross inspection indicated that they had not passed through the "glass eel" stages.

All of the migrants were apparently recent arrivals from offshore waters. According to Bertin (1957) stage V of the European eel is the true invading form of coastal waters. Smith (1913) pointed out that American elvers have little pigment upon arrival, but brown pigmentation is quickly acquired, and they develop the coloration of adults by the time they penetrate as far inland in the Potomac as Washington.

Total lengths as small as 49.0 mm also signify recent arrival from offshore waters. The smallest elvers found by Hildebrand and Schroeder (1928) in Chesapeake Bay and by Herman (1958) in Narragansett Bay measured 48.0 mm.

The occurrences reported here plus the winter records in Table 2 indicate that a relatively small percentage of migrating immature American eels regularly find their way into temperate estuaries several months in advance of the main spring and summer groups. Schmidt (1925) obtained full grown leptocephali off the New England coast toward the end of the year. Individuals are, therefore, in position to metamorphose and become winter forerunners of the mass landward migration. Wheatland (1956), considering the records of Nichols and Breder (1926) for estuaries of the New York area, stated that an elver taken on January 27, 1953, in Long Island Sound appeared later than usual and "may have been the product of an earlier spawning." Few reported cap-

tures in local waters during winter may be due to the infrequency of intensive night sampling programs. The Rhode Island specimens were taken at night and the first Raritan Bay elver, found on a beach early in the morning, had presumably been stranded by the fall of the evening tide. On the basis of replicate catches at consecutively later hours Deelder (1952) concluded that swimming activity of European elvers is greatest at night, and during the day they are dug into the bottom in shallow areas or stationary in deeper water.

Water temperatures during the time of year when elvers arrive in New England and Middle Atlantic estuaries range from values near the winter minimum. Throughout Raritan Bay the temperature at the bottom was less than 2.3°C for seven and a half weeks prior to the first capture. Herman (personal communication) found elvers near frozen areas in the northern portion of Narragansett Bay at -0.8°C. Occurrences at such low temperatures contrast with Deelder's (1952) observation that a temperature of about 4.5°C is quite possibly required before immature European eels start migration into brackish water; the journey was interrupted one year when the water cooled from more than 5°C in February to 2.0°C in March. Further information may demonstrate that elvers reaching American shores have lower limiting temperatures than those which swing across the Atlantic to Europe, or, more simply, American populations are less sensitive to low temperature.

Occurrences during the coldest portion of the year are significant in the light of Hiyama's (1952) experiments, criticized by Deelder (1953), showing a reversible thermotaxis for the elver of the Japanese eel; it tends to swim into cooler water when temperature is low (about 11°C) and leaves water at 14°C to enter still warmer water. During winter, decreasing water temperatures are encountered as one moves into a temperate estuary from offshore, and it may be that the gradient attracts or helps to orient winter forerunners on their landward journey.

Finally, winter occurrences of elvers in brackish waters may denote arrival of first spawned individuals. On the other hand, local currents may sweep a segment of the population landward. If so, annual variations in arrival dates and numbers of winter forerunners should have importance in the study of offshore currents.

REFERENCES

Bertin, L. 1957. Eels. A Biological Study. Philosophical Library Inc., New York, 192 pp.

Bigelow, H. B., and W. C. Schroeder. 1953. Fishes of the Gulf of Maine. 1st revision. Fish Wildl. Serv. Fish. Bull., **53**: 1–577.

Deelder, C. L. 1952. On the migration of the elver (*Anguilla vulgaris* Turt.) at sea. J. du Cons., **18**: 187–218.

———. 1953. Review of: Thermotaxis of eel fry in stage of ascending river mouth, by Y. Hiyama; J. du Cons., **19**: 105.

Fish, C. J. 1925. Seasonal distribution of the plankton of the Woods Hole region. Bull. U.S. Bur. Fish., **41**: 91–179.

Fowler, H. W. 1906. The fishes of New Jersey. Ann. Rep. of the New Jersey State Museum (1905), **2**: 35–477.

Herman, S. S. 1958. The planktonic fish eggs and larvae of Narragansett Bay. MS. University of Rhode Island

Hildebrand, S. F. and W. C. Schroeder. 1928. Fishes of Chesapeake Bay. Bull. U.S. Bur. Fish. (1927), **43**: 1–366.

Hiyama, Y. 1952. Thermotaxis of eel fry in stage of ascending river mouth. Japanese J. Ichthyol., **2**: 23–30. (In Japanese).

Nichols, J. T. and C. M. Breder. 1926. The marine fishes of New York and southern New England. Zoologica, N.Y., **9**: 1–192.

Schmidt, J. 1925. The breeding places of the eel. Ann. Rep. Smithsonian Inst. (1924): 279–316.

Smith, H. M. 1913. The mysterious life of the common eel. Natl. Geogr. Mag., **24**: 1140–1146.

Strubberg, A. 1913. The metamorphosis of elvers as influenced by outward conditions. Medd. Komm. Hav. Fisk., **4**(3): 1–11.

Tucker, D. W. 1959. A new solution to the Atlantic eel problem. Nature, **183**: 495–501.

Wheatland, S. B. 1956. Oceanography of Long Island Sound, 1952–1954. VII. Pelagic fish eggs and larvae. Bull. Bingham Oceanogr. Coll., **15**: 234–314.

Harry P. Jeffries

Narragansett Marine Laboratory
University of Rhode Island
Kingston, Rhode Island

A Technique for Separating Nematodes and Small Arthropods from Marine Muds[1]

While studying the nematodes and other small inhabitants of the mud of the Georgia salt marshes it was necessary to remove the animals quantitatively from the mud. A number of methods have been worked out for removing small animals from terrestrial soils (Caveness and Jensen 1955; Overgaard 1948) and for removing larger animals from aquatic muds (Anderson 1959). These were not suitable for nematodes and other animals of similar size in marine muds without modification.

Picking over, under the low-power microscope, the residue remaining in a sieve of 145 or 65μ openings can result in a fairly good separation of the microfauna. Unfortunately, this is very time consuming unless the animals are the size of small annelids.

The use of a funnel apparatus (Overgaard 1948) would not work with the mud as it came from the core, as it was too dense for the animals to move through easily and they did not fall into the collecting bottles. The samples were accordingly sieved before being placed on the screen in the funnel. This made the sample sufficiently loose for the animals to move easily and be successfully extracted. However, the animals of the Georgia marshes are adapted to withstand quite high temperatures and it was necessary to run the extraction temperature gradually up to 38°C in order to get adequate results. With such a high temperature, some animals were inactivated before they had moved out of the sample. Also the method was time consuming. This would have been offset by the advantage of getting all sizes of animals if the preliminary sieving were not necessary, but since the smaller forms were lost through the sieve anyway, the funnel method was abandoned.

Centrifuging the sample without preliminary treatment (Caveness and Jensen 1955) did not work since the particles of the marine sediments were much finer than the terrestrial soil particles for which the technique was developed. The animals were not separated from this fine matter and were very difficult to sort out.

The following technique proved satisfactory. Cores were taken with an aluminum tube 1 cm^2 in area and immediately cut into 2 cm long sections to insure that there could be no error from movement of the animal within the core. The core was then mixed with water and washed through a sieve of 65μ openings. The residue on the sieve was layered on a 6–7 cm deep layer of white Karo syrup, in a plastic centrifuge tube, balanced with another tube and spun for 15 min at 2600 rpm in an International model SBV, giving about 1100 g relative centrifugal force. It was found that Karo gave excellent results and saved the work of making the special solution used by Caveness and Jensen (1955).

After centrifugation the syrup and water were poured off into a beaker, diluted to a suitable consistency, and poured through a sieve with 40μ openings to catch the animals. The animals were then washed into a Syracuse watch glass for identification and counting. Sea water was used throughout.

The animals were in remarkably good condition after this procedure. Copepods, mites, and other small arthropods were usually unharmed. About half of the nematodes appeared quite normal while the rest were motionless or coiled tightly. Even a few of the small annelids survived the process. Most of the coiled nematodes could be straightened by heating gently on a hot plate overnight. They were then treated with formalin, and placed in a solution of 1 part glycerine to 3 parts distilled water which was subsequently heated to evaporate the water. In this way samples were processed from collection to glycerine mounts in 2 days.

Since all the smallest animals were lost in the first sieving the water, about 250 ml, was saved. After stirring, three 1/10 aliquots

[1] Supported by National Science Foundation Grant G-6156. Contribution No. 13 from Marine Institute, University of Georgia, Sapelo Island, Georgia.

Table 1

Type of substrate	Nematodes extracted Number	% of total	Small arthropods extracted % of total
Dense marsh mud, about equal clay and silt	35	95%	—
"	112	100%	—
"	134	85%	89%
Very soft mud, clay and silt	143	90%	100%
Soft mud, clay and silt	282	93%	100%
Firm mud with 70% sand	117	100%	100%

were placed in Syracuse watch glasses, allowed to settle, and the animals therein counted. In this way all the animals in the sample were accounted for.

If the core contained appreciable amounts of large plant detritus, the sample was first washed through a coarse-meshed sieve (8 mesh/cm). A jet from a wash bottle was sufficient to force all the animals through the sieve. If the sample contained appreciable sand it was found that nematodes would stick to the grains, be carried down through the Karo and be lost. To remedy this, the sand was removed by shaking the sample in a test tube, allowing the sand to settle and pouring the water with the animals through the sieve. This was repeated several times to ensure complete removal of the animals.

The method was tested by extracting the animals and then carefully examining the residue in the bottom of the centrifuge tube under a microscope to find how many individuals were missed. The results of several such tests are presented in Table 1. It can be seen that the method gives about 90% or better extraction from a variety of marsh substrates.

John M. Teal
Institute of Oceanography
Dalhousie University, Halifax, N.S.

References

Anderson, R. O. 1959. A modified flotation technique for sorting bottom fauna samples. Limnol. & Oceanogr., **4**: 223–225.

Caveness, F. E. and H. J. Jensen. 1955. Modification of the centrifugal-flotation technique for the isolation and concentration of nematodes and their eggs from soil and plant tissue. Proc. Helm. Soc. Washington, **22**: 87–89.

Overgaard, C. 1948. An apparatus for quantitative extraction of nematodes and rotifers from soil and moss. Natura Jutlandica, **1**.

Stabilization of the Carbohydrate Content of Sea Water Samples

It is the practice during many oceanographic investigations to analyze sea water samples immediately after collection. This has been necessary due to difficulties involved in stabilizing a sample during storage. Collier and Marvin (1953) have demonstrated that the inorganic to organic phosphorus ratio of a sea water sample can be effectively stabilized for long periods of time by freezing. The purpose of this experiment was to determine if carbohydrate materials (see Collier, *et al.* 1953) could be similarly stabilized.

A sea water medium that contained a bacterized culture of the organism *Prorocentrum* sp. was chosen because such a medium is relatively rich in dissolved organic material. A portion of the medium was first centrifuged through a Sharples centrifuge to remove as many organisms as possible and was then thoroughly mixed. Fifty-ml portions were then dispensed into clean 25 × 200 mm culture vials and capped with solid polyethylene screw caps. All cap-glass junctions were sealed with plastic insulating tape. Of the 130 vials filled, half were frozen and stored at minus 18°C and half were stored at room temperature. Groups of 8 vials were taken from both the frozen and unfrozen material at the intervals indicated in Figure 1. Employing the method of Zein-Eldin and May (1958), four measurements of the carbohydrate content of each sample were made, making a total of 32 determinations per time interval.

Immediately prior to storage, the carbohydrate concentration in the centrifuged sea water medium was estimated at 3.8 ±

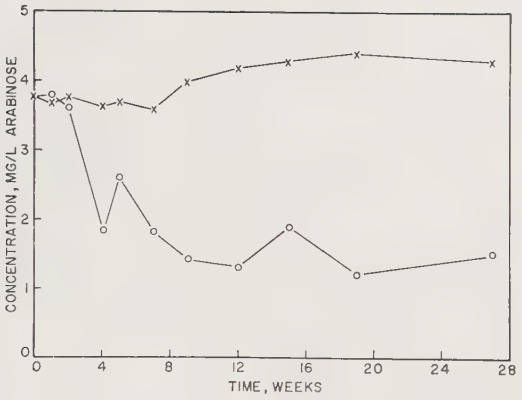

FIG. 1. Carbohydrate content of sample plotted against storage time of sample. The two plots x—x—x and 0—0—0 indicate mean values of 8 frozen and unfrozen samples respectively at each time interval.

0.1 mg/L arabinose equivalents. Subsequent estimates from the frozen and unfrozen material are plotted against time in Figure 1. Estimates from the frozen material did not vary more than ± 0.15 mg/L for a period of 7 weeks. In contrast, carbohydrate concentration in the material not frozen generally diminished as length of storage time increased. The somewhat erratic values obtained in the medium not frozen might be expected since biotic activity within different vials undoubtedly proceeds at different rates.

Comparison of the two curves shows that freezing stabilizes the carbohydrate content of sea water for periods up to 7 weeks. Since the average deviation within groups of frozen samples was less than 0.1 mg/L at any one period (Table 1), a reasonable assumption is that the increase in mean values after the 7th week was not due to sampling variation, but rather to unknown factors. For example, bacteria or fungi not eliminated during the process of centrifugation could have survived the freezing temperatures. The growth of these organisms might have caused the increase of arabinose equivalents.

TABLE 1. *Standard error of means of each frozen group*

Group	Elapsed time Weeks	Mean (mg/L)	Standard error (mg/L)
1	1	3.70	0.096
2	2	3.80	0.082
3	4	3.65	0.047
4	5	3.70	0.043
5	7	3.65	0.057
6	9	4.0	0.032
7	12	4.2	0.038
8	15	4.3	0.030
9	19	4.4	0.078
10	27	4.3	0.083

This supposition suggests that further investigation should be conducted on the biological activities of micro-organisms at freezing temperatures and during the time lapse between thawing and analysis of samples.

BILLIE Z. MAY
U.S. Bureau of Commercial Fisheries,
St. Petersburg Field Station,
St. Petersburg, Florida

REFERENCES

COLLIER, A. W. AND K. T. MARVIN. 1953. Stabilization of the phosphate ratio of sea water by freezing. U.S. Fish and Wildl. Serv., Fish. Bull., **79:** 71–76.

COLLIER, A. W., S. M. RAY, A. W. MAGNITSKY AND J. O. BELL. 1953. Effect of dissolved organic substances on oysters. U.S. Fish and Wildl. Serv., Fish. Bull., **84:** 166–185.

ZEIN-ELDIN, Z. P. AND B. Z. MAY. 1958. Improved n-Ethylcarbazole determination of carbohydrates with emphasis on sea water samples. Analyt. Chem., **30:** 1935–1941.

A NOTE ON A STABILIZED STARCH INDICATOR FOR USE IN IODOMETRIC AND IODIMETRIC DETERMINATIONS

The problem of preventing biotic hydrolysis of aqueous starch indicators has stimulated innumerable searches for preservatives capable of sustained anti-biotic effect. Walton (1928) has listed abstracts of twenty-three methods purporting to prevent hydrolysis for periods ranging from two months to three years. A more recent and

widely-used method is that of Hillebrand and Lundell described by Pierce and Haenisch (1948 p. 237). The method uses 1 g of boric acid per 100 ml of indicator solution.

At this laboratory we have tried several of the recommended methods with varying degrees of success and obtained the most favorable results with a modification of the method described by Hillebrand and Lundell (Pierce and Haenisch 1948). We have increased the starch concentration to 5% and have protected the stock bottle and the dispensing bottle from light. Under these conditions, hydrolysis was inhibited in the stock bottle for periods ranging from one to six months. However, in the dispensing bottle in which the contents were repeatedly exposed to the atmosphere, deterioration was rapid and the supply had to be frequently replaced.

The reliability of such solutions is not satisfactory where mass production techniques are required. In addition we have one or more isolated insular stations where iodometric work is being performed by persons without chemical training and using limited facilities. Our goal then, has been to find a starch indicator simple to prepare, economical to use, and free from hydrolytic deterioration for at least a year and preferably longer.

Experience with different methods of preserving aqueous suspensions indicated that the most satisfactory way to obtain the stability we desired was to work with a nonaqueous medium, which in itself is an unfavorable ecological medium, rather than to rely on toxic additives to suppress biotic activity in a highly favorable growth medium. A modified starch glycerite was considered. Referring to Walton (1928) again, we find that he catalogued eight abstracts covering the reactions of heated glycerine and starch. In these and subsequent publications there is a great diversity of opinion (frequently conflicting) as to the reaction(s) involved and the nature of the product(s) of such reactions. The following résumé concerning the formation of soluble starch illustrates the ambiguities in the literature:

A) Walton (1928 p. 11)

Starch $\xrightarrow[\text{glycerin}]{\text{heat}}$ soluble starch $\xrightarrow{}$ (blue iodine reac.)

hexa saccharide $\xrightarrow{}$ Trisaccharide

(red iod. reac.) (no iod. reac.)

B) Grant (1944 p. 802)

Starch $\xrightarrow[\text{glycerin}]{\text{heat}}$ Sol starch (amylodextrin)

(yellow iod. reac.)

C) Ibid.

Starch $\xrightarrow[\text{glycerin}]{\text{heat}}$ Starch glycerite

D) Bernthsen (1941 p. 372)

1. Starch cold, dil $\xrightarrow[\text{acid}]{}$ soluble starch
2. Starch $\xrightarrow[\text{glycerin}]{\text{heat}}$ soluble starch
3. Starch hydrolysis $\xrightarrow{}$ amylodextrin $\xrightarrow{}$ (blue iod. reac.)

erythrodextrin $\xrightarrow{}$ achroodextrin

(red iod. reac.) (no iod. reac.)

E) Gregory (1942 p. 602)

Starch $\xrightarrow[\text{glycerin}]{\text{heat}}$ soluble starch

(amylodextrin)

The reader will note the contradictory statement concerning the color reaction of amylodextrin and the ambiguity of the references to starch glycerite and soluble starch. Until we understand the above reactions and can compare the spectral profiles of the reaction products, the term *modified starch glycerite* will be used arbitrarily in reference to the soluble starch-glycerin indicator described herein.

The indicator is prepared as follows:

1. Add 3 g of powdered soluble starch to 100 ml of glycerin and heat to 190°C.
2. Remove from heat and cool to room temperature. Developing turbidity may be ignored as it does not interfere with the efficacy of the indicator.

If the solution should gel with age reheat to 190°C and dilute with small amount of glycerin. Use 1 to 2 drops per 100 ml sample.

This indicator has been in use at this and other laboratories for approximately eight months. The solutions have been exposed to conditions favorable to deterioration such as exposure to light, air, etc. To date, there has been no indication of any deterioration. The color developed with a drop of the indicator is an intense blue with a sharp end point.

JOHN W. VAN LANDINGHAM
U.S. Bureau of Commercial Fisheries
Honolulu, Hawaii

REFERENCES

BERNTHSEN, A. 1941. A Textbook of Organic Chemistry. Revised by J. J. Sudborough. D. Van Nostrand, New York. 1371 pp.

GRANT, J. 1944. Hackh's Chemical Dictionary. Ed. 3, Blakiston, Philadelphia. 925 pp.

GREGORY, T. C. 1942. The Condensed Chemical Dictionary. Ed. 3, Reinhold, New York. 755 pp.

PIERCE, W. C. AND HAENISCH, E. L. 1948. Quantitative Analysis. Ed. 3, Wiley, New York. 520 pp.

WALTON, R. P. 1928. A Comprehensive Survey of Starch Chemistry. **1**. Chemical Catalogue Co., New York. 604 pp.

A World List of Students of Rotifiers

The "Second Preliminary List of the World's Rotifer Students" has been prepared by Dr. John J. Gallagher. He will be pleased to distribute copies to all interested persons who write to him at: 1716 East Terry, Pocatello, Idaho.

BOOK REVIEWS

KUSNEZOW, S. L. 1959. **Die Rolle der Mikroorganismen im Stoffkreislauf der Seen.** (Translated from the Russian by Dr. Alfred Pochmann.) x + 301 pp. VEB Deutscher Verlag der Wissenschaften, Berlin. DM36 (= approx. $8.65).

The book by Kuznetsov published in 1952 has been virtually unavailable outside the Soviet Union, and hence the present translation is most welcome. It is more than a translation, however, in that Kuznetsov has generously attempted to add and integrate many items of research published since 1952. Hence, in reality the present book is a second edition of the original. The translation is very readable, unambiguous, and presumably accurate in all details, since Kuznetsov himself is competent in the German language and no doubt worked closely with Pochmann.

One of our big deficiencies in teaching and understanding limnology in the western countries has been the general lack of knowledge concerning aquatic bacteriology and particularly the absence of a summary of what is known in this field. The Russians, and particularly Kuznetsov, have been more active in this area than any other group, and to have the results of their investigations finally available in a generally accessible summary should do much to stimulate further investigation on aquatic bacteria. The need for an increased exchange of information between the East and the West is demonstrated not only by our unawareness of much that has been done in Russia, but also by the apparent unawareness by the Russians, if Kuznetsov can be considered representative, of much that has gone on in the western European countries and in North America. A few specific examples will be cited below.

The book begins with a consideration of general limnology, especially those aspects affecting the activities of bacteria. After a description of the methods used in the study of aquatic bacteria and their spatial distribution in water and sediments, Kuznetsov discusses the factors responsible for the oxygen dynamics in lakes and then considers in detail the biogeochemical cycles of carbon, nitrogen, sulphur, and iron. Phosphorus is not treated in detail. The final two brief chapters concern the use of radioisotopes in studying the involvement of microorganisms in the biogeochemical cycles, and the possibility of increasing the significance to man of natural waters through the manipulation of these cycles. Not only are references made to the Russian papers in these various areas, but considerable amounts of the actual data are presented.

In the field of bacteriology, Kuznetsov apparently is aware of and has a good grasp of the world literature, but in his description and understanding of limnological processes there appear to be many deficiencies. Particularly in the field of North American limnology one has the feeling that much of Kuznetsov's information is derived second hand from Welch's text on limnology rather than from the original sources.

The few obvious errors or deficiencies in statements of general limnology do not detract from the overall value of the book in detailing the role of bacteria in aquatic processes. For example, in Table 1 the temperature of maximum density at 90 m is listed as 3.5°C, and in the discussion of light transmission only suspenoids are listed as a controlling factor.

Meromictic lakes are called bioanisotropic lakes, based on work published by Yegunov in 1900, which greatly antedates the work published by Findenegg on Carinthian lakes in the 1930's. (Incidentally, the work of Findenegg in this area is not mentioned.) A few lakes, such as Belovod and Chornoye, that have had detailed bacteriological studies made on them are of this type.

Much of the dissolved organic matter in water is unavailable to bacteria as a nutritional substrate. The assimilable matter in oligotrophic lakes is only 2–3%, in a dystrophic lake 1.5%, and even in eutrophic lakes only up to 15%. Likewise, in the sediments much of the organic matter is unassimilable by bacteria. Kuznetsov suggests that the organic matter that can be hydrolyzed by 5% H_2SO_4 approximates the quantity utilizable by bacteria.

Changes with depth of organic and inorganic constitutents in lake sediments are interpreted as the result of bacterial activity alone. Changes in the depositional environment of a lake as affecting the chemistry of the sediments are not mentioned.

The best technique for determining the total bacterial population of water or sediments is by direct count. Even in eutrophic lakes only about 0.1% of the bacteria enumerated by direct count can be induced to grow on agar plates. This cannot be attributed to a large proportion of dead cells, since Razumov and Peshkov have demonstrated that only about 10% of the bacteria present momentarily are dead.

The species of bacteria in aquatic habitats are very incompletely known. Many of them are probably oligocarbophiles or autotrophs, which cannot be readily cultured in the laboratory. Kuznetsov and coworkers obtained 119 species of saprophytes from 30 lakes. Greatest significance seems to lie not in the percentage composition of the population but in the ratio between spore formers and non-spore formers in relation to the trophic level, being lowest in eutrophic lakes. Spore formers are said to increase proportionately to the degree of mineralization of organic matter. Actinomycetes,

which evidently play a very great role in the organic cycles, have scarcely been investigated.

The numbers of bacteria in water are much greater than ordinarily realized by limnologists. During summer stagnation even in oligotrophic lakes (*e.g.* Baikal) the population of the surface waters is about 100,000/ml. In mesotrophic lakes about 1 million cells per ml occur, in eutrophic lakes about 3 million, and in the salt lakes of western Siberia as high as 13 million. The highest counts are generally observed towards the end of summer stagnation or during fall overturn. Calculations of the biomass of bacteria in various lakes on a wet weight basis indicate a variation from ⅕ the biomass of the phytoplankton in Lake Beloye to a biomass equal to that of the phytoplankton in Lake Dolgoye. In Baikal the bacteria amount to ⅓ the biomass of the phytoplankton.

Likewise in lake sediments the bacteria are very abundant, generally several hundred million to more than a billion bacterial cells per gram wet weight of surface sediments. The numbers decrease with depth in the sediments, showing a marked decrease below 0.6 m, although on the basis of direct counts there are always large numbers present, even in the basal clays of Lake Piyavochnoye. This is in contrast to studies based on culturing techniques, which quite generally show a logarithmic decrease with depth. Such tremendous numbers of bacteria substantiate what we have already known regarding the great importance of these organisms in the biogeochemical cycles.

In the discussion of the oxygen dynamics of lakes there are several curious deficiencies in Kuznetsov's presentation, showing that the East has been as isolated from the West as the reverse. For example, the various types of oxygen stratification are described, without mention of the work of Åberg and Rodhe in this area. Furthermore, Kuznetsov's description of the utilization of oxygen in the hypolimnion seems somewhat naïve. He regards the hypolimnion as being essentially a quiet mass of water, with the only currents being the density currents resulting from the cooling of the water in contact with the sediments. This denser water with its reduced oxygen content then flows down grade to the bottoms of the depressions, resulting in the relatively rapid reduction in oxygen content usually observed in deep waters. Although Alsterberg's theory of horizonal density cell circulation may not be strictly correct, recent evidence based on part on internal seiches has demonstrated that the hypolimnion is far from a stationary mass of water.

Reduction of the oxygen content of deep water is brought about almost entirely by bacterial activity. Oxygen utilization by purely chemical means is negligible, and probably even completely lacking in oligotrophic lakes. Respiration by animals is also of minor importance. In Lake Beloye, for example, zooplankton accounted for only 2% of the oxygen utilized. Oxygen utilization by bacteria can result both from direct bacterial respiration and from oxidation of CH_4 and H_2 by the particular autotrophs involved. In Lake Beloye the latter accounted for about half of the total oxygen utilization.

I have presented some of these details to indicate the great wealth of information contained in this book, the general awareness of which should do much to stimulate work on aquatic bacteria in other parts of the world. Especially now when we are experiencing great activity in the study of the energetics and dynamics of aquatic ecosystems, it is essential to understand the role of the bacteria in these processes. The other point made in this review is that there are differences between the East and West in the understanding of fundamental limnological processes, which obviously must be resolved.

Ecologists in general will find this a stimulating book to read. We are presently using it as a sourcebook in production limnology.

David G. Frey

Indiana University
Bloomington, Indiana

Emery, K. O. 1960. **The Sea Off Southern California,** A modern habitat of Petroleum. 366 pp. John Wiley & Sons, New York. $12.50.

In few parts of the world has there been such intensive investigation of the sea and its life as in the waters adjacent to southern California, the home waters of Scripps Institution of Oceanography and the Allan Hancock Foundation. The region concerned is roughly that bounded on the north and east by the coast of California from Pt. Conception to Descanso Pt., by the 32nd parallel on the south and the 121st meridian on the west. Although this region is complex and perhaps atypical on a world wide basis, so much of the work done here has been cited in textbooks that many of its features will be recognized by students of several disciplines.

At the present time an average of 150 papers concerning this region are published annually, and the bibliography of this book includes nearly 900 titles. It is the author's expressed purpose to bring this material together and to "integrate" the basic knowledge that has accumulated, especially in the post-war years. Consequently little attention is given to sampling methods and techniques. The subject matter of the various sections is extraordinarily diverse, as might be expected. There are seven such sections, each subdivided into various topics: Physiography, Lithology, Structure, Water, Life, Sediments, and Economic Aspects. Under Physiography we find a brief history of exploration, a description of shoreline types, of basins, troughs and submarine canyons, and so on. Lithology includes such topics as rocks, phosphorite and paleogeography, and the section on Structure

is also geological in content. The section on water includes most of the material of oceanography; currents, waves, tides and paleooceanography. The section on life is a brief review of the characteristic organisms of the various environmental types from the intertidal zone to the bathyal regions, and is summarized by an interesting attempt to compute the organic budget (pp. 175–179). Since the author is primarily interested in sediments, it is natural to expect a more detailed treatment of this subject, and 116 pages are devoted to it as contrasted with 40 pages for biology and 41 for oceanography. Organic matter in sediments is given considerable attention, however, and pollution problems and fisheries are treated in the final section on economic aspects. Still, the treatment is somewhat out of balance (if the reviewer were to compile such a book, the sedimentologist would say that there was too much biology in it), and quite a few of the statements about biology are somewhat naïve and some of the wording is about equivalent to confusing such terms as outcrop and anticline. The section on economic aspects also includes such matters as shipwrecks, landslides, beach preservation and offshore drilling for petroleum. The discussion of pollution, especially of the disposal of radioactive wastes, is especially useful.

In all, the author has done a remarkable job of summarizing, which will be of value to students in helping them realize the complex nature of oceanography, and to economic geologists, engineers and other professional people who need a handy reference. In evaluating a work such as this, which covers so many different subjects, the reviewer is in much the same position as the author: he must take some things on faith. In this case the quality of treatment is surprisingly high. The subtitle, "a habitat of petroleum" is debatable; at least no biologist would consider an inanimate substance, even of organic origin, as having a habitat, but this does serve to emphasize the similarities between the existing region and the geological structures which are a major source of petroleum. The attempt to compute the organic budget is at best a sporting try, as the author fully realizes; nevertheless lumping the wet weights of all types of bottom organisms produces misleading figures, and the assumption of various efficiencies throws the budget even further off. Obviously much more critical work remains to be done, and if this treatment stimulates it, the author can congratulate himself.

On the whole the book is clearly written, although at times the use or lack of use of commas requires closer reading to follow the meaning, and "however" is a bit overused. There are some typographical errors, but not too many for a book this size (the double column format makes the book at least a third larger than the number of pages would indicate). The illustrations are abundant and well chosen, but a few of them are a bit too small. A reprint of the map of submarine topography previously published by the G S A is included at the end of the book. The price is comparatively low for a book by this publisher, perhaps because a large sale is anticipated. Since the book will be useful to many, the publisher's expectations should be fulfilled.

JOEL W. HEDGPETH

Pacific Marine Station,
Dillon Beach, California